Document Title

Summary of the toxicological studies for Prothioconazole + Spiroxamine EC 460 (160+300 g/L)

Data Requirement(s)

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

Document MCP

Section 7: Toxicological studies

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

Date

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Author(s)

[redacted]

ERM
On behalf of Bayer AG
Crop Science Division

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Version history

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

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

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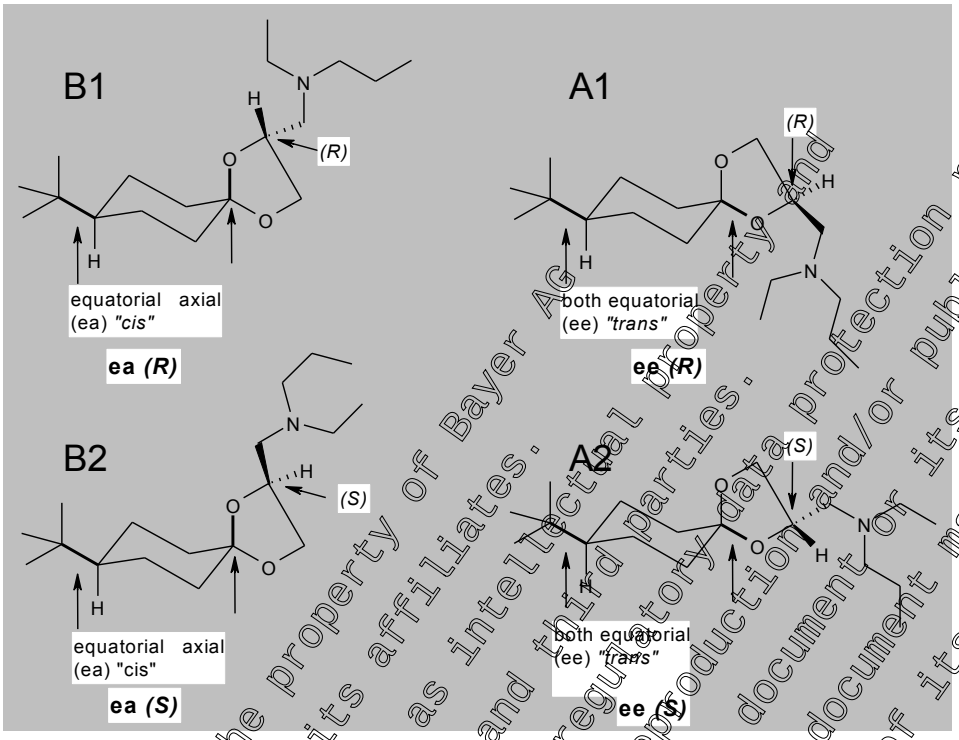
CP 7 TOXICOLOGICAL STUDIES ON THE PLANT PROTECTION PRODUCT

Spiroxamine was included in Annex I to Council Directive 91/414/EEC in 1999 (Directive 1999/73/EC, Entry into Force on 1 September 1999). This Supplementary Dossier contains data which were not submitted at the time of the Annex I inclusion of spiroxamine under Council Directive 91/414/EEC and which were therefore not evaluated during the first EU review. However, all studies submitted for the first renewal of spiroxamine have also been summarised according to current guidance and included in the dossier. Where studies meet relevant validity criteria, new robust study summaries have been provided in the appropriate dossier section. However, where studies do not meet relevant validity criteria and are not considered acceptable, less detailed summaries may have been provided alongside discussions of study deficiencies. All relied upon study reports are submitted in Document K for this second renewal of approval dossier or in Document K for the first renewal submissions.

All data which were already submitted by Bayer AG (former Bayer CropScience) for the Annex I inclusion and first renewal under Council Directive 91/414/EEC are contained in the draft Re-Assessment Report (RAR) 2010 and its revised RAR 2017, and are included in the Baseline Dossier provided by Bayer AG.

The formulation Prothioconazole + Spiroxamine EC 460 (160+300 g/L), abbreviation PTZ + SPX EC 460, is an emulsifiable concentrate formulation containing 160 g/L of prothioconazole and 300 g/L of spiroxamine. This formulation is registered throughout Europe under trade names such as HELIX, IMPULSE GOLD, INPUT 460 EC, INPUT CLASSIC, KROTOM, PROLINE MAX 460 EC, Prosaro Plus, ROMBUS POWER, THESORUS, THESORUS 460 EC. PTZ + SPX EC 460 was already a representative formulation of Bayer AG for the first renewal of Spiroxamine under Council Directive 91/414/EEC.

Spiroxamine consists of four isomers (two diastereomers each with its corresponding two enantiomers which are in a 1:1 ratio) as shown in the schematic below. The isomer nomenclature presented in some historical documentation may differ with respect to the A/B and corresponding trans/cis notation as a result of a discrepancy in referencing, which is discussed in detail in position paper M-761468-01-1 (see CA 1.7/01). It is recommended that the stereo assignments depicted here, together with the A and B notation should be used exclusively going forward to ensure continuity of information throughout the dossier.



CP 7.1 Acute toxicity

The acute oral toxicity study confirmed Prothioconazole + Spiroxamine EC 460 (160+300 g/L) to be of low to moderate toxicity with LD_{50} values of >500 mg/kg bw and <100 mg/kg bw for male and female rats, respectively. The dermal toxicity study confirmed Prothioconazole + Spiroxamine EC 460 (160+300 g/L) to be of low toxicity, with an LC_{50} >4000 mg/kg bw. A four hour nose-only acute inhalation toxicity study confirmed Prothioconazole + Spiroxamine EC 460 (160+300 g/L) to be of low toxicity, with an LC50 value of 2.221 mg/L (equivalent to 399.8 mg/kg bw).

Prothioconazole + Spiroxamine EC 460 (160+300 g/L) was found to be irritant in a primary skin irritation study undertaken in the rabbit and deemed to cause serious, but reversible eye damage in the eye irritancy test in the rabbit.

A skin sensitisation study, employing the maximization method confirmed Prothioconazole + Spiroxamine EC 460 was not a skin sensitiser.

Therefore, Prothioconazole + Spiroxamine EC 460 (160+300 g/L) does not warrant classification for acute dermal toxicity or skin sensitisation, with classification required for acute oral (Acute Tox. Cat. 4, H302), inhalation (Acute Tox. Cat. 4, H332), skin irritancy (Skin Corrosion/Irritation, Cat. 2, H315) and serious eye irritant (Eye Damage/Irritation, Cat. 2, H319) endpoints according to the harmonised classification Regulation 1272/2008.

Table CP 7.1-1: Acute toxicity studies with PTZ + SPX EC 460

Type of study	Species	Results	Classification (Annex I for Regulation (EC) 1272/2008)	Annex CP Point / Reference
Oral route	Rat	LD_{50} ♂: >500 mg/kg bw LD_{50} ♀: <1000 mg/kg bw	Acute Tox. Cat. 4, H302	CP 7.1.1/01 M-087810-02-1
Dermal route	Rat	LD_{50} ♂+♀: >4000 mg/kg bw	Insufficient for classification	CP 7.1.2/01 M-087804-02-1
Inhalation route	Rat	LD_{50} 4 h ♂+♀: 2.221 mg/L (399.8 mg/kg bw)	Acute Tox. Cat. 4, H332	CP 7.1.3/01 M-035481-01-1
Skin irritation	Rabbit	Marked inflammatory reactions that were reversible by day 14	Skin Corrosion/Irritation, Cat. 2, H315	CP 7.1.4/01 M-083125-01-1
Eye irritation	Rabbit	Serious irritant reactions that were reversible by day 19	Eye Damage/Irritation, Cat. 2, H319	CP 7.1.5/01 M-083107-01-1
Skin sensitisation	Guinea pig	Skin sensitiser (Maximization method)	Insufficient for classification	CP 7.1.6/01 M-066247-01-1

CP 7.1.1 Oral toxicity

Data Point:	KCP 7.1.1/01
Report Author:	[redacted]
Report Year:	2002
Report Title:	JAU 6476 160 EC & KWG 4168 300 (c.n.: --; Spiroxamine) - Study for acute oral toxicity in rats
Report No:	31560
Document No:	M-087810-02-1
Guideline(s) followed in study:	OECD 423; Directive 67/548/EEC, Annex IV B, Part B, B.1 tris; US-EPA 712-C-98-190, OPPTS 870.1100
Deviations from current test guideline:	None
Previous evaluation:	yes, evaluated and accepted RAR (2010)
GLP/Officially recognised testing facilities:	Yes, conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	Yes

Executive Summary

The acute oral toxicity of JAU 6476 160 EC & KWG 4168 300 (Prothioconazole + Spiroxamine EC 460) was investigated in a study on the rat performed to GLP and OECD 423 (1996). Groups of Wistar rats (3/sex) received a single oral gavage dose of Prothioconazole + Spiroxamine EC 460 at dose levels of 0 (vehicle controls) and 200 and 500 mg/kg bw for male rats and 200, 500 and 2000 mg/kg bw for female rats and were observed for 14 days. The test article was formulated in demineralised water and administered orally via gavage employing a dose volume of 10 mL/kg bw.

Mortalities were observed in all female rats within 2 days of dosing at 2000 mg/kg bw. Clinical signs were observed in both sexes at 500 g/kg bw within 3 hours of dosing and comprised of decreased motility. At 2000 mg/kg bw, clinical signs were reflective of CNS type effects (including but not limited to decreased motility, uncoordinated gait, lateral positions, spasmodic states, laboured breathing and increased salivation) observed in females within 1 hour of dosing. All surviving animals gained weight during the study period.

Gross necropsy of decedents revealed abnormalities including general autolysis and discolouration of the liver, spleen and kidneys. Animals sacrificed at the end of the post-treatment observation period showed no evidence of test article-related gross pathological changes.

Under the conditions of this study, the acute oral LD_{50} of Prothioconazole + Spiroxamine EC 460 was calculated to be >500 mg/k bw for males and <1000 mg/kg bw in female rats. Therefore, according to Annex I for Regulation (EC) 1272/2008, Prothioconazole + Spiroxamine EC 460 EC is classified as Acute Toxicity (Oral) Category 4, H302 (harmful if swallowed).

Materials and methods

A. Materials

1. Test Material: Prothioconazole + Spiroxamine EC 460 (alternative name: JAU 6476 160 EC & KWG 4168 300)

Description:	Clear dark-yellow liquid
Lot/Batch No.:	06920/0045(0019)
Purity:	160.4 g/L (prothioconazole); 296.2 g/L (spiroxamine)
CAS No.:	178928-70-6 (prothioconazole); 118134-30-8 (spiroxamine)
Stability:	Confirmed stable for the duration of the study (expiry date: 2 November 2001)

2. Vehicle and/or positive control: Demineralised water/not applicable

3. Test animals:

Species: Rat

Strain:	Wistar (SPF, HsdCpb:WU)
Age at dosing:	♂: 8-10 wks; ♀: 9 wks
Weight at dosing:	♂: 231-307 g; ♀: 190-208 g
Source:	Harlan Winklemann GmbH, Borchen, District of Paderborn
Acclimation period:	At least 5 days
Diet:	NAFAG No. 9441 W 10, *ad libitum* (except for 17 hours before and 2 hours after dosing)
Water:	Municipal water, *ad libitum*
Housing:	Group housed (3/sex/cage)
4. Environmental conditions:	
Temperature:	22 ±2ºC
Humidity:	55 ±5%
Air changes:	*ca*. 10/h
Photoperiod:	12 hours light/dark cycle

B. Study Design

1. In life dates:	23 August to 13 September 2001 (experimental dates)
2. Animal assignment and treatment:	After an acclimatisation period of *ca*. 5 days, rats were pre-arranged based on weight classes and allocated to groups by computer-based stratified random sampling. After being fasted for *ca*. 17 hours, rats (3/sex/gp) were administered the test article by a single oral *via* gavage, employing a dose volume of 10 mL/kg bw, for the following doses: ♂: 200 and 500 mg/kg bw; ♀: 200, 500 and 2000 mg/kg bw. The rats were fasted for a further 2 hours post administration before being allowed to feed. The animals were then observed for a period of 14 days.
3. Statistics:	Not undertaken. For body weight, the mean value and standard deviation were calculated.

C. Methods:

1. Homogeneity and achieved concentration analysis of the dose:	Not performed.
2. Observations:	Appearance and behaviour were recorded several times on the day of treatment and at least once a day thereafter for 14 days.
3. Body weights:	Body weights were recorded on Study Day 1 (prior to dosing), day 8 thereafter and at test termination.
4. Food consumption:	Not recorded.
5. Sacrifice and pathology:	Organs/tissues were examined macroscopically. No histopathological analysis was undertaken.

Results and Discussion

A. Homogeneity and achieved concentration analysis:

Not undertaken. Analyses for achieved concentration, homogeneity or stability of test article formulations were not conducted as part of this study, as this is not a requirement of the regulatory test guidelines.

B. Observations:

1. Clinical signs of toxicity:	Clinical signs were reflective of CNS toxicity, were observed in both sexes within 3 hours of dosing and included decreased motility. At 2000 mg/kg bw, clinical signs were observed in ♀ within 1 hour of dosing and included

	decreased motility, uncoordinated gait, lateral positions, spasmodic states, laboured breathing and increased salivation.
2. Mortality:	Mortalities were observed in all ♀ rats within 2 days of dosing at a dose level of 2000 mg/kg bw. Refer to Table CP 7.1.1/01-1.

C. Body weight and food consumption:

1. Body weight:	Body weight gain was not affected during the post-treatment observation period in either sex. Refer to Table CP 7.1.1/01-1.
2. Food consumption:	Not measured.

Table CP 7.1.1/01-1-: Overview of acute oral toxicity in rats treated with Prothioconazole + Spiroxamine EC 460 (160+300 g/L): mortality and body weight

Parameter	♂ (mg/kg bw)						♀ (mg/kg bw)								
	200			500			200			500			2000		
Overall mortality[a]	0/3			0/3			0/3			0/3			3/3		
Day	1	8	15	1	8	15	1	8	15	1	8	15	1	8	15
Mortality[a]	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3	3/3	-/-	-/-
Body weight (g) ±s.d.	305 ±2.0	352 ±6.7	373 ±7.5	240 ±8.1	292 ±12.6	321 ±12.8	196 ±6.6	223 ±6.9	231 ±10.1	204 ±3.5	228 ±4.2	239 ±6.1	202 ±5.2	-	-
Net body weight gain (g)	68 ±8.5			82 ±4.9			35 ±3.5			35 ±3.5			n/a		
Acute oral LD_{50}	>500 mg/kg bw						>1000 mg/kg bw								

a Mortality: no. of animals found dead / no. of animals treated

D. Necropsy:

Macroscopic examination of decedent rats revealed abnormalities including general autolysis and discolouration of the liver, spleen and kidneys. Animals sacrificed at the end of the post-treatment observation period showed no evidence of test article-related gross pathological changes.

E. Deficiencies:

None.

Assessment and conclusion by applicant:

Assessment: This study is deemed acceptable and meets the requirements in 284/2013.

Conclusion: Under the conditions of this study, the acute oral LD$_{50}$ of Prothioconazole + Spiroxamine EC 460 was calculated to be >500 mg/k bw for males and >1000 mg/kg bw in female rats. Therefore, according to Annex I for Regulation (EC) 1272/2008, Prothioconazole + Spiroxamine EC 460 EC is classified as Acute Toxicity (Oral) Category 4, H302 (harmful if swallowed).

CP 7.1.2 Dermal toxicity

Data Point:	KCP 7.1.2/01
Report Author:	[redacted]
Report Year:	2002
Report Title:	JAU 6476 160 EC & KWG 4168 300 (c.r.s. --; Spiroxamine) - Study for acute dermal toxicity in rats
Report No:	31562
Document No:	M-087804-02-1
Guideline(s) followed in study:	OECD 402; US-EPA 712-C-98-192, OPPTS 870.1200; Directive 67/548/EEC Annex V, Part B.3.
Deviations from current test guideline:	None
Previous evaluation:	yes, evaluated and accepted RAR (2010)
GLP/Officially recognised testing facilities:	Yes, conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	Yes

Executive Summary

The acute dermal toxicity of JAU 6476 160 EC & KWG 4168 300 (Prothioconazole + Spiroxamine EC 460) was investigated in a study in rats performed to GLP and OECD 402 (1987). Prothioconazole + Spiroxamine EC 460 was applied to the shorn dorsal skin of Wistar rats (5/sex/group) at a dosage level of 4000 mg/kg bw (both sexes). Rats were observed for 15 days.

All animals survived to the scheduled necropsy. Local effects at the site of application were evident in both genders at 4000 mg/kg bw with partial reddening of the treatment site/partial encrusting/induration and additionally in females partial scale was observed. Local effects were observed from Study Day 2 to 8 in males and from Study Day 2 to termination in females. No clinical signs were observed in female rats; reactivity was decreased in males on Study Day 4. Body weight gains were slightly impaired compared to expected gains in females on Study Day 8, however these had returned to normal by the end of the study.

All animals were subject to gross necropsy. Animals sacrificed at the end of the post-treatment observation period showed no evidence of test article-related gross pathological changes.

Under the conditions of this study, the acute dermal LD_{50} of Prothioconazole + Spiroxamine EC 460 was found to be >4000 mg/kg bw in male and female rats. Therefore, according to Annex I for Regulation (EC) 1272/2008 the formulation requires no obligatory labelling requirement for acute dermal toxicity and is unclassified.

Materials and methods

A. Materials

1. Test Material:	Prothioconazole + Spiroxamine EC 460 (alternative name: JAU 6476 160 EC & KWG 4168 300)
Description:	Clear dark-yellow liquid
Lot/Batch No.:	06920/0045(0019)
Purity:	160.4 g/L (prothioconazole), 296.2 g/L (spiroxamine)
CAS No.:	178928-70-6 (prothioconazole), 118134-30-8 (spiroxamine)
Stability:	Confirmed stable for the duration of the study (expiry date: 2 November 2001)
2. Vehicle and/or positive control:	None/not applicable
3. Test animals:	
Species:	Rat
Strain:	Wistar (SPF HsdCpb:Wu)
Age at dosing:	♂: 9 wks; ♀: 11 wks
Weight at dosing:	♂: 246-261 g; ♀: 210-232 g
Source:	Harlan Winklemann GmbH, Borchen, District of Paderborn
Acclimation period:	At least 5 days
Diet:	NAFSG No: 9441 W 10, *ad libitum*
Water:	Municipal water, *ad libitum*
Housing:	Individually housed
4. Environmental conditions:	
Temperature:	22 ±2°C
Humidity:	55 ±5%
Air changes:	At least 10/h
Photoperiod:	12 hours light/dark cycle

B. Study Design

1. In life dates:	23 August to 06 September 2001 (experimental dates)
2. Animal assignment and treatment:	After an acclimatisation period of *ca*. 5 days, rats were pre-arranged based on weight classes and allocated to groups by computer-based stratified random sampling. An area of the dorsal skin was shaved before application (area of 6.0 x 5.0 cm). On the day of application, Prothioconazole + Spiroxamine EC 460 was weighed out onto a gauze dressing (comparable in size to the shaved test site) at a dose level of 4000 mg/kg bw, which was then applied to the test site and secured in place with tape. After 24 hours of exposure, the dressings were removed and the treated skin site cleaned with soap and water. The animals were then observed for a period of 15 days.
3. Statistics:	Not undertaken. For body weight, the mean value and standard deviation were calculated.

C. Methods:

1. Homogeneity and achieved concentration	Not performed.

analysis of the dose:

2. Observations: Appearance and behaviour was recorded several times on the day of treatment and at least once a day thereafter for 15 days.

3. Body weights: Recorded on Study Day 1 (prior to dosing) and weekly thereafter.

4. Food consumption: Not recorded.

5. Sacrifice and pathology: Organs/tissues were examined macroscopically. No histopathological analysis was undertaken

Results and Discussion

A. Homogeneity and achieved concentration analysis:

Not undertaken. Analyses for achieved concentration, homogeneity or stability of test article formulations were not conducted as part of this study as this is not a requirement of the regulatory test guidelines.

B. Observations:

1. Clinical signs of toxicity: Local effects were observed from Study Days 2 to 8 in ♂ rats and from Study Day 2 to test termination in ♀ rats. No clinical signs were observed in ♀ rats, reactivity was decreased in ♂ rats on Study Day 4. Refer to Table CP 7.1.2/01-01.

2. Mortality: No mortalities occurred during the study. Refer to Table CP 7.1.2/01-01.

Table CP 7.2.1/01-1-: Overview of acute dermal toxicity in rats treated with Prothioconazole + Spiroxamine EC 460 (160+300 g/L): mortality and body weight

Parameter	♂ (mg/kg bw)			♀ (mg/kg bw)		
	2000			4000		
Overall mortality[a]	0/3			0/3		
Day	1	8	15	1	8	15
Mortality[a]	0/3	0/3	0/3	0/3	0/3	0/3
Body weight (g) ±s.d.	252 ± 5.7	290 ± 9.6	308 ± 8.5	218 ± 8.7	215 ± 10.7	230 ± 13.2
Net body weight gain (g)	50 ±7.6			14 ±8.5		
Acute oral LD_{50}	>4000 mg/kg bw			>4000 mg/kg bw		

a Mortality: no. of animals found dead / no. of animals treated

C. Body weight and food consumption:

1. Body weight: Body weight gains were slightly impaired in ♀ on Study Day 8, however these had returned to normal by the end of the study.

2. Food consumption: Not measured.

D. Necropsy:

Animals sacrificed at the end of the post-treatment observation period showed no evidence of test article-related gross pathological changes.

E. Deficiencies:

None.

Assessment and conclusion by applicant:

Assessment: This study is deemed acceptable and meets the requirements in 284/2013.

Conclusion: Under the conditions of this study, the acute dermal LD_{50} of Prothioconazole + Spiroxamine EC 460 was found to be >4000 mg/kg bw in male and female rats. Therefore, according to Annex I for Regulation (EC) 1272/2008 the formulation has no obligatory labelling requirement for acute dermal toxicity and is unclassified.

CP 7.1.3 Inhalation toxicity

Data Point:	KCP 7.1.3/01
Report Author:	[redacted]
Report Year:	2002
Report Title:	JAU 6476 160 EC & KWG 4168 300 - Study on acute inhalation toxicity in rats according to OECD No. 403
Report No:	31735
Document No:	M-035481-01-1
Guideline(s) followed in study:	OECD 403; Directive 92/69/EEC, Method B.2.; US-EPA 712C-98-193, OPPTS 870.1300
Deviations from current test guideline:	None
Previous evaluation:	yes, evaluated and accepted RAR (2010)
GLP/Officially recognised testing facilities:	Yes, conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	Yes

Executive Summary

The acute inhalation toxicity of Prothioconazole + Spiroxamine EC 460 was investigated in a study in rats performed to GLP and OECD 403 (1981). Groups of Wistar rats (5/sex) were exposed nose only for a single 4 hour period to a liquid atmosphere (deemed mist) to a mean achieved aerosolised concentrations of 1018 and 4505 mg/m^3, with MMAD ±GSD of 1.3 ±1.71 and 1.7 ±1.9, respectively obtained for the aerosol size distribution, with >80% of the inhalable fraction <3 µm. The observation period was 14 days post-exposure.

Clinical signs of toxicity manifest as CNS-type effects were reported (including but not limited to piloerection and ungroomed fur, reduced motility, tremors) for animals in the 1018 mg/m^3 and above. For surviving animals, all were free of clinical signs at day 14. Statistically significant reductions in rectal temperature were observed in animals from the 1018 mg/m^3 dose group and in males 4805 mg/m^3. As female treated at 4805 mg/m^3 died during the exposure, no assessment of body temperature could be made.

Deaths were restricted to animals dosed at 4805 mg/m^3 (4 males, 5 females), occurring during exposure (females) or 3 days post exposure (males).

A transient reduction in the body weights was noted on day 3 in animals from the 1018 mg/m^3, with recovery thereafter. For males treated at 4805 mg/m^3, body weight gain was reduced during the 14 day recovery period.

Under the conditions of this study the rat acute inhalation 4 hour nose only LC_{50} Prothioconazole + Spiroxamine EC 460 of is 2.221 mg/L in males and females (equivalent to 399.8 mg/kg bw). Therefore, according to Annex I for Regulation (EC) 1272/2008 the formulation is termed as a mist due to its liquid form and classified under Acute Toxicity (Inhalation) in Category 4, H332 (harmful if inhaled).

Materials and Methods

A. Materials:

1. Test Material:	Prothioconazole + Spiroxamine EC 460 (alternative name: JAU 6476 160 EC & KWG 4168 300)
Description:	Clear dark-yellow liquid
Lot/Batch No.:	06920/0045(0019)
Purity:	160.4 g/L (prothioconazole); 296.2 g/L (spiroxamine)
CAS No.:	178928-70-6 (prothioconazole); 118134-30-8 (spiroxamine)
Stability of test compound:	Confirmed stable for the duration of the study (expiry date: 2 November 2001)
2. Vehicle and/or positive control:	None/not applicable
3. Test animals:	
Species:	Rat
Strain:	Hsd CpB:WU (SPF)
Age at dosing:	*ca.* 8 wks
Weight at dosing:	♂: 173-198g; ♀: 165-183g
Source:	Harlan-Winkelmann, Borchen, District of Paderborn
Acclimation period:	At least 5 days
Diet:	Altromin® 1324 diet for rats and mice, *ad libitum* (except during treatment)
Water:	Municipal water, *ad libitum*
Housing:	Housed individually
4. Environmental conditions:	
Temperature:	22 ±2°C
Humidity:	*ca.* 50%
Air changes:	*ca.* 10/h
Photoperiod:	12 hour light/dark

B Study Design:

1. In life dates:	8 October 2001 to 24 October 2001 (experimental dates)
2. Animal assignment and treatment:	Following acclimatisation rats were randomly assigned to the test groups. Groups of rats (5 ♂♂) were exposed (nose only) for 4 hours to atmospheres containing Prothioconazole + Spiroxamine EC 460 (aerosol) at gravimetric concentrations of 0 (vehicle control), 1017.5 or 4805 mg/m^3. The observation period was 14 days post-exposure.
3. Generation of the test atmosphere/chamber description:	During the 4 hour exposure period, rats were housed individually in plexiglass exposure tubes (following a period of acclimatisation prior to dosing). Prothioconazole + Spiroxamine EC 460 at target concentrations of 0, 1000 and 5000 mg/m^3 was automatically injected into a baffle with compressed air (air that has had water, dust and oil removed). This mixture was then pumped into the inhalation chamber (volume: *ca.* 20 L). The baffle increased the efficiency of aerosol generation, whilst also removing larger particles. The air flows (15 L/minute) were continuously monitored with rotameters and re-adjusted to the nominal settings where necessary. Air samples were taken on four occasions, at hourly intervals. Determination of the concentration of Prothioconazole + Spiroxamine EC 460 in the test atmosphere was determined by the analysis of the airborne concentration of the active ingredient. After sampling, the adsorbents were eluted and the analyst was determined. Temperature and air humidity in the exposure chamber were measured over 5 minute intervals. Particle size distribution analysis were taken from the

immediate vicinity of the breathing zone and analysis performed by means of a Berner cascade impactor. The impactor media were gravimetrically evaluated.

4. Statistics: Mean values and simple standard deviations were calculated for the body weights. more frequent findings for the respiratory tract were evaluated using Fisher's Pairwise Test with a preceding RxC chi square test

C. Methods:

1. Observations: Test animals were several times on the day of the exposure, then twice daily (morning and evening). They were also assessed at weekends. The animals were only assessed while they were in the tubes if there were clear signs occurring such as spasms, abnormal movements, and severe dyspnea. An assessment of their reflexes was also undertaken.
Rectal temperatures were taken at the end of treatment.

2. Body weights: The body weights of the rats were recorded manually before exposure, and on day 3 and 7 of the post-treatment observation period, and then weekly thereafter.

3. Food consumption: Not recorded.

4. Sacrifice and pathology: All animals were sacrificed post-treatment and subjected to a gross necropsy.

Results and Discussion

A. Atmospheric data:

Findings indicate that particles were well within the respirable range.

Table CP 7.1.3/01-1: Overview of acute inhalation toxicity study in rats treated with Prothioconazole + Spiroxamine EC 460 (160+300 g/L): exposure parameters of the acute inhalation toxicity

Parameter		Value	
Dose group (nominal mg/m³)		1000	5000
Mean achieved atmosphere concentration (mg/m³)		1018	4805
Mean achieved atmosphere concentration (mg/L)		1.018	4.805
Dose group (internal dose mg/kg bw/d)[a]		183.2	864.9
Chamber flow rate (L/min)		15	15
Particle size (MMAD ± GSD)		1.3 ±1.71	1.7 ±1.9
Aerosol mass <3 µm (%)		94.1	81.3.
Chamber air temperature (°C)	During exposure	22	22
Relative humidity (%)	During exposure	<5%	
Air changes (/h)	During exposure	Not detailed	
O_2 conc. (%)	During exposure	Not detailed	
CO_2 conc. (%)	During exposure	Not detailed	

a Internal dose (mg/kg bw) = inhalation dose (mg/L) *x* 45 L/kg bw/h (rat respiration rate) *x* 4 h (daily inhalation exposure) *x* 1 (default respiratory absorption: 100%). No further correction considered necessary [taken from SANCO 7531-rev.10]

B. Observations:

1. Clinical signs: 0 mg/m³: no clinical signs of toxicity were evident.
1018 mg/m³ (1183.2 mg/kg bw): clinical signs of toxicity manifest as CNS type effects were reported (including bradypnea, laboured breathing pattern, reduced movement, nasal discharge, reddened nostrils, piloerection).
4805 mg/m³ (864.9 mg/kg bw): for ♂, clinical signs of toxicity were similar to that already reported, but with increased severity (stridor, high-legged gait, corneal opacity, exophthalmos, tremor, prostration, choreoathetotic

	convulsions). A ♀ rat died during exposure, therefore no clinical signs were reported. For surviving animals, all were free of clinical signs at day 14. ♂ from the high dose group displayed changed in reflexes (decreased grip strength tonus, corneal reflex, impaired righting response).
2. Mortality	Deaths were restricted to animals dosed at 4805 mg/m³ (4♂, 5♀), occurring during exposure (♀) or 3 days post exposure (♂). Refer to Table CP 7.1.3/01-2.
3. Rectal temperatures:	Statistically significant reductions in rectal temperature were observed in animals from the 1018 mg/m³ dose group and in ♂ 4805 mg/m³. As 5 rats treated at 4805 mg/m³ died during the exposure, no assessment of body temperature could be made.

Table CP 7.1.3/01-2:Overview of acute inhalation toxicity study in rats treated with Prothioconazole + Spiroxamine EC 460 (160+300 g/L): mortality and body weight

Parameter	♂ (actual concentration (mg/m³) [target mg/m³])												♀ (actual concentration (mg/m³) [target mg/m³])											
	0				1018 [1000]				4805 [5000]				0				1018 [1000]				4805 [5000]			
Overall mortality[a]	0/5				0/5				4/5				0/5				0/5				5/5			
Day	0	3	7	14	0	3	7	14	0	3	7	14	0	3	7	14	0	3	7	14	0	3	7	15
Mortality[a]	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	5/5	4/5	0/1	0/1	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	5/5	-	-	-
Body weight (g) ±s.d	197.0 ±6.6	209.8 ±7.5	237.6 ±9.7	274.6 ±14.3	182.6 ±6.3	176.1 ±10.4	204.2 ±11.3	237.2 ±16.3	188.0 ±6.4	141.0 ±n/a	148.0 ±n/a	133.0 ±n/a	179.0 ±7	184.6 ±2.3	191.6 ±2.6	204.0 ±5.5	171.6 ±4.3	163.6 ±4.5	172.6 ±6.8	192.6 ±5.9	180.4 ±6.0	-	-	-
Net body weight gain (g)	77.6 ±13.9				54.6 ±13.6				-58 ±n/a				25 ±5.6				21 ±2.6				-			
Rectal temp. (°C) at end of treatment	37.9				31.2**				26.4**				38.2				31.9**				-			
Acute oral LC_{50}	2212mg/m³ (2.221 mg/L), equivalent to 399.8 mg/kg bw																							

a Mortality: no. of animals found dead / no. of animals treated

C. Body weight and food consumption:

1. Body weight:	A transient reduction in the body weights was noted on day 3 in animals from the 1018 mg/m^3, with recovery thereafter. For ♂ treated at 4805 mg/m^3, body weight gain was reduced during the 14 day recovery period.
2. Food consumption:	Not measured

D. Necropsy:

Animals which died during exposure had exhibited nose: red discolouration; lung: partial collapse, dark-red discolorations; lung oedema (trachea with foamy content); stomach and remaining gastrointestinal-tract: bloated; foamy, red to yellowish mucus in lumen; corneal opacity.

Animals sacrificed at the end of the observation period had no evidence of concentration related changes in the lungs or other organs.

E. Deficiencies:

None

Assessment and conclusions by applicant:

Assessment: This study is deemed acceptable and meets the requirements in 284/2013.

Conclusion: Under the conditions of this study the rat acute inhalation 4 hour nose only LC_{50} for Prothioconazole + Spiroxamine EC 460 is 2.221 mg/L in males and females (equivalent to 399.8 mg/kg bw). Therefore, according to Annex I for Regulation (EC) 1272/2008 the formulation is termed as a mist due to its liquid form and classified under Acute Toxicity (Inhalation) in Category 4, H332 (harmful if inhaled).

CP 7.1.4 Skin irritation

Data Point:	KCP 7.1.4/01
Report Author:	[redacted]
Report Year:	2001
Report Title:	Acute skin irritation test (patch test) of JAU 6476 160 EC & KWG 4168 300 in rabbits
Report No:	R8083
Document No:	M-083125-01-1
Guideline(s) followed in study:	OECD 404; EC guideline B.4
Deviations from current test guideline:	Yes Whilst it is recognised under the current guidance and the requirements of (EU) 284/2013 that a tiered testing strategy should be followed with a validated in vitro test method, this approach has not been adopted. However, the study was conducted prior to the publication of the EU commission regulation and validation of acceptable in vitro alternatives. These in vivo data are however considered valid to address this endpoint.
Previous evaluation:	yes, evaluated and accepted RAR (2010)
GLP/Officially recognised testing facilities:	Yes, conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	Yes

Executive Summary

In a primary dermal irritation study, 3 male Himalayan rabbits were dermally exposed to 0.5 mL of Prothioconazole + Spiroxamine EC 460 applied to an area of shaven dorsal skin measuring an area of approximately 6 cm x 6 cm for 4 hours using a semi-occlusive patch. The application sites were observed at 1, 24, 48, 72 hours and then daily until day 14 after patch removal with erythema/eschar and oedema formation scored. Irritation was scored according to the assessment criteria for primary skin irritation (Draize scale).

Prothioconazole + Spiroxamine EC 460 applied neat caused erythema (grade 1) which was observed in all animals at 24 h after patch removal and persisted until day 11 in one animal and day 12 in the other two.

Oedema (grade 1) was evident in two animals 48 h after patch removal and persisted until 72 h after patch removal.

Induration of the skin (*i.e.* thickening of the skin, resulting from oedema /inflammation) was observed on day 4, turning to laceration of the skin on day 5 through to 6, peeling on day 7 to 8 and finally partly reddened and thickened on day 9 though to 12 in one animal, persisting to day 13 in the remaining two.

Under the conditions of this study, Prothioconazole + Spiroxamine EC 460 caused dermal irritation that was reversible by day 14. Therefore, according to Annex I for Regulation (EC) 1272/2008, Prothioconazole + Spiroxamine EC 460 is classified as Skin Corrosion/Irritation, Category 2, H315 (causes skin irritation).

Materials and Methods

A. Materials:

1. Test Material:	Prothioconazole + Spiroxamine EC 460 (alternative name: JAU 6476 160 EC & KWG 4168 300)
Description:	Clear dark-yellow liquid
Lot/Batch No.:	06920/0045(0019)
Purity:	160.4 g/L (prothioconazole); 296.2 g/L (spiroxamine)
CAS No.:	178928-70-6 (prothioconazole); 118134-30-8 (spiroxamine)
Stability of test compound:	Confirmed stable for the duration of the study (expiry date: 2 November 2001)
2. Vehicle and/or positive control:	Not applicable/not applicable
3. Test animals:	
Species:	Rabbit
Strain:	Himalayan
Age at dosing:	*ca.* 3.5 months
Weight at dosing:	♂: 2.1-3.0 kg
Source:	LPT Laboratory of Pharmacology and Toxicology KG, Wankendorf
Acclimation period:	At least 20 days
Diet:	Altromin 2023, *ad libitum*
Water:	Municipal water, *ad libitum*
Housing:	Individually housed
4. Environmental conditions:	
Temperature:	Not provided
Humidity:	Not provided
Air changes:	Not provided

Photoperiod: Not provided

B. Study Design:

1. **In life dates:** 13 September 2001 to 27 September 2001 (experimental dates)
2. **Animal assignment and treatment:** Approximately 24 hours before test article application fur was clipped (area: 6 cm *x* 6 cm) from the dorso-lateral area of the trunk of each of three rabbits. On day of application 0.5 mL of the test article was applied (as supplied, undiluted) to the test site and a gauze patch applied. The patches were held in place with semi-occlusive dressing for the duration of the exposure period, 4 hours. At the end of the exposure period patches were removed and the exposed skin areas were carefully washed with water. The contralateral skin area not treated with test article served as control. For each animal, the Draize scale was used to assess skin irritation at 1, 24, 48, 72 hours and then daily until day 14 after patch removal with erythema/eschar and oedema formation scored.
3. **Evaluation criteria:** Primary irritation index (Draize scale):

Erythema and eschar formation

- No erythema	0
- Very slight erythema	1
- Well-defined erythema	2
- Moderate to severe erythema	3
- Severe erythema to slight eschar formation	4

Oedema formation

- No oedema	0
- Very slight oedema	1
- Slight oedema	2
- Moderate oedema	3
- Severe oedema	4

4. **Statistical analysis:** Not undertaken

C. Methods:

1. **Homogeneity and achieved concentration analysis of the dose:** Not undertaken.
2. **Observations:** The application sites were observed at 1, 24, 48, and 72 h after patch removal according to the Draize scoring system for skin irritation/corrosion. As there was an irritant effect to the skin of the animals, they were also assessed daily from day 4 to 14.
3. **Body weights:** Animals were weighed on the day of application.
4. **Food consumption:** Not recorded.
5. **Sacrifice and pathology:** Not undertaken.

Results and Discussion

A. Homogeneity and achieved concentration analysis:

Not undertaken. Analyses for achieved concentration, homogeneity or stability of test article formulations were not conducted as part of this study, as this is not a requirement of the regulatory test guidelines.

B. Observations:

1. **Clinical signs of toxicity:** None noted.
2. **Mortality:** No animals died in the study.

3. Skin irritation: Erythema (grade 1) was observed in all animals at 24 h after patch removal and persisted until day 11 in one animal and day 12 in the other two.
Oedema (grade 1) was evident in two animals 48 h and after patch removal and persisted until 72 h after patch removal.
Induration of the skin (*i.e.* thickening of the skin, resulting from oedema, inflammation) was observed on day 4, turning to laceration of the skin on days 5 through to 6, peeling on day 7 to 8 and finally partly reddened and thickened on day 9 though to 12 in one animal, persisting to day 13 in the remaining two.
By day 13 one animal had recovered with on evidence of oedema or erythema with the remaining two animals recovered by day 14.

Table CP 7.1.4/01-1: Summary of skin irritation scores according to the Draize scheme: Individual and mean skin irritation

Time point (post patch removal)	Erythema			Oedema		
	Animal number					
	1	2	3	1	2	3
1 h	0	0	0	0	0	0
24 h (Day 1)	1	1	1	0	0	0
48 h (day 2)	1	1	1	1	1	0
72 h (day 3)	1	1	1	1	1	0
4 days	1	1	1	0[a]	0[a]	0[a]
5 days	1	1	1	0[b]	0[b]	0[b]
6 days	1	1	1	0[b]	0[b]	0[b]
7 days	1	1	1	0[c]	0[c]	0[c]
8 days	1	1	1	0[c]	0[c]	0[c]
9 days	1	1	1	0[d]	0[d]	0[d]
10 days	1	1	1	0[d]	0[d]	0[d]
11 days	1	1	1	0[d]	0[d]	0[d]
12 days	0	1	1	0[d]	0[d]	0[d]
13 days	0	0	0	0	0[d]	0[d]
14 days	-	0	0	-	0	0
Mean (24 – 72 h)	1.0			0.7		

- not examined
a induration of the skin
b laceration of the skin
c peeling of skin
d new skin partly reddened and indurated

C. Body weight and food consumption:

1. Body weight: Animals were only weighed at the beginning of the study, thus effects on body weight cannot be assessed.

2. Food consumption: Not applicable.

D. Necropsy:

Not undertaken.

E. Deficiencies:

Whilst it is recognised under the current guidance and the requirements of (EU) 284/2013 that a tiered testing strategy should be followed with a validated *in vitro* test method, this approach has not been adopted. However, the study was conducted prior to the publication of the EU commission regulation and validation of acceptable *in vitro* alternatives. These *in vivo* data are however considered valid to address this endpoint.

Assessment and conclusions by applicant:

Assessment: Study meets the current guidance and the requirements in 284/2013.

Conclusion: Under the conditions of this study, Prothioconazole + Spiroxamine EC 460 caused dermal irritation that was reversible by day 14. Therefore, according to Annex I for Regulation (EC) 1272/2008, Prothioconazole + Spiroxamine EC 460 is classified as Skin Corrosion/Irritation Category 2, H315 (causes skin irritation).

CP 7.1.5 Eye irritation

Data Point:	KCP 7.1.5/01
Report Author:	[redacted]
Report Year:	2001
Report Title:	Acute eye irritation study at JAU 6476 160 EC & KWG 4168 300 by installation into the conjunctival sac of rabbits
Report No:	R8084
Document No:	M-083107-01-1
Guideline(s) followed in study:	OECD 405, EC-guideline B.5
Deviations from current test guideline:	Yes Whilst it is recognised under the current guidance and the requirements of (EU) 284/2013 that a tiered testing strategy should be followed with a validated in vitro test method, this approach has not been adopted. However, the study was conducted prior to the publication of the EU commission regulation and validation of acceptable in vitro alternatives. These in vivo data are however considered valid to address this endpoint.
Previous evaluation:	yes, evaluated and accepted RAR (2010)
GLP/Officially recognised testing facilities:	Yes, conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	Yes

Executive Summary

In a primary eye irritation study, 0.1 ml Prothioconazole + Spiroxamine EC 460 was instilled into the right eye of 3 male Himalayan rabbits. Eyelids were held to together for ~1 second to prevent loss of material. The other eye served as a control. For each animal, the score on the Draize scale was assigned at 1, 24, 48, 72 hours, 7 and 14 days. The areas of the eye assigned in this way were the cornea (opacity and area affected), iris (hyperaemia, reaction to light), conjunctivae - *i.e.* conjunctiva of bulbus, lids, and nictitating membrane (erythema, chemosis), discharge and aqueous humour (opacity). In addition any serious lesions or toxic effects other than ocular ones were recorded.

Corneal opacity

- animal #1: 24 and 48 h (grade 3), 72 h to 7 days (grade 2), 8 to 19 days (grade 1) after instillation;
- animal #2: 24 and 48 h (grade 3), 72 h and 4 days (grade 2), 5 to 11 days (grade 1) after instillation;
- animal #3: 24 and 48 h (grade 3), 72 h (grade 2) and 4 to 1 5 days (grade 1) after instillation.

The fluorescein test performed 24 hrs after instillation revealed corneal staining in all animals (whole surface). The fluorescein test performed 7 days after instillation revealed corneal staining in animal # and #3 (½ the surface) and animal #2 (¼ of the surface). The fluorescein test performed 14 days after instillation revealed corneal staining in animal nos. 1 and 3 (¼ of the surface).

Irritation of the iris (grade 1) was observed in all animals 24 h to 7 days after instillation, in animal #1 until 19 days and in animal #3 until 8 days after instillation.

Conjunctival redness

- all animals (grade 1 to 3) observed 1 hour to 8 days after instillation.

Conjunctival chemosis

- all animals (grade 1 to 3) observed 1 to 72 h after instillation, in animal #2 until 5 days after instillation.

In addition, whitish deposits (likely pus) was observed in animal #1 48 h to 5 days after instillation.

Under the conditions of this study the test article, Prothioconazole + Spiroxamine EC 460 showed irreversible eye damage. According to Annex I for Regulation (EC) 1272/2008, Prothioconazole + Spiroxamine EC 460 is classified as Eye Damage/Irritation, Category 1, H318 (causes serious eye damage).

Materials and Methods

A. Materials:

1. Test Material:	Prothioconazole + Spiroxamine EC 460 (alternative name: JAU 6476 160 EC & KWG 4168 300)
Description:	Clear dark-yellow liquid
Lot/Batch No.:	06920/0045(0019)
Purity:	160.4 g/L (prothioconazole); 296.2 g/L (spiroxamine)
CAS No.:	178928-70-6 (prothioconazole); 118134-30-8 (spiroxamine)
Stability of test compound:	Confirmed stable for the duration of the study (expiry date: 2 November 2001)
2. Vehicle and/or positive control:	Deionised water/not applicable
3. Test animals:	
Species:	Rabbit
Strain:	Himalayan
Age at dosing:	*ca.* 3.5 months
Weight at dosing:	♂: 2.3-2.7 kg
Source:	LPT Laboratory of Pharmacology and Toxicology KG, Wankendorf
Acclimation period:	At least 20 days
Diet:	Altromin 2023, *ad libitum*
Water:	Municipal water, *ad libitum*
Housing:	Individually housed
4. Environmental conditions:	
Temperature:	Not provided
Humidity:	Not provided
Air changes:	Not provided
Photoperiod:	Not provided

B. Study Design:

1. In life dates:	23 September 2001 to 14 October 2001 (experimental dates)
2. Animal assignment and treatment:	The lower eyelid of each rabbit was gently pulled to expose the eyeball, then 0.1 mL of the test article was applied to the conjunctival sac of the right eye of each of the rabbits. The eyelids were then gently held together for a second to limit the loss of material. The other eye of each rabbit served as a control. For each animal, the score on the Draize scale was assigned at 1, 24, 48, 72 hours, 7 and 14 days. The areas of the eye assigned in this way were

the cornea (opacity and area affected), iris (hyperaemia, reaction to light), conjunctivae - *i.e.* conjunctiva of bulbus, lids, and nictitating membrane (erythema, chemosis), discharge and aqueous humour (opacity). In addition any serious lesions or toxic effects other than ocular ones were recorded.

3. Evaluation criteria:

Eye irritation:

Cornea

Opacity: degree of density:

- No ulceration or opacity 0
- Scattered or diffuse areas of opacity details of iris clearly visible 1
- Easily discernible translucent area, details or iris slightly obscured 2
- Nacreous area, no details or iris visible, size of pupil barely discernible 3
- Completely opaque cornea, iris not discernible through the opacity 4

Iris:

- Normal 0
- Markedly deepened rugae, congestion, swelling, moderate, circumcorneal hyperaemia, or injection 1
- No reaction to light, haemorrhage, gross destruction 2

Conjunctivae:

Erythema:

- Blood vessels normal 0
- Some blood vessels definitely hyperaemic 1
- Diffuse, crimson colour, individual vessels not easily discernible 2
- Diffuse, beefy redness 3

Chemosis:

- No swelling 0
- Any swelling above normal (includes nictitating membranes) 1
- Obvious swelling with partial eversion of lids 2
- Swelling with lids about half closed 3
- Swelling with lids more than half closed 4

Discharge

- No discharge 0
- Slightly increased discharge 1
- Discharge with slight moistening of periorbital areas 2
- Discharge with considerable moistening of periorbital areas. 3

4. Interpretation criteria:

Slight irritation:

- Cornea opacity 1.00 – 1.99
- Hyperaemia of iris, reaction to light ≥0.5
- Erythema of conjunctivae 1.00 – 2.49
- Chemosis 1.00 – 1.99

Changes persisting for more than 24 hours, reversible within 7 days or less

Moderate irritation:

- Cornea opacity 2.00 – 2.99
- Hyperaemia of iris, reaction to light 1.00 – 1.50
- With 3 animals used 1.00 – 1.99
- Erythema of conjunctivae ≥2.5
- Chemosis ≥2.0

Changes persisting for more than 24 hours, reversible within 14 days or less

Severe irritation:

Moderate irritation, however reversible within 21 day or less

Corrosive:

- Cornea opacity	≥3.0
- Hyperaemia of iris, reaction to light	>1.5
- With 3 animals used	= 2.0

Or other significant tissue destruction that persist or are expected to persist for 21 days or more

C. Methods:

1. Homogeneity and achieved concentration analysis of the dose:	Not undertaken.
2. Observations:	The application sites were observed at 1, 24, 48, 72 h, 7, 14 and 21 days post application both grossly and using a slit lamp and scored for local reactions using the Draize eye irritation test.
3. Body weights:	Animals were weighed on the day of application.
4. Food consumption:	Not recorded.
5. Sacrifice and pathology:	Not undertaken.

Results and Discussion

A. Homogeneity and achieved concentration analysis:

Not undertaken. Analyses for achieved concentration, homogeneity or stability of test article formulations were not conducted as part of this study, as this is not a requirement of the regulatory test guidelines.

B. Observations:

1. Clinical signs of toxicity:	None noted.
2. Mortality:	No animals died in the study.
3. Eye irritation:	*Corneal opacity* - animal #1: 24 and 48 h (grade 3), 72 h to 7 days (grade 2), 8 to 19 days (grade 1) after instillation; - animal #2: 24 and 48 h (grade 3), 72 h and 4 days (grade 2), 5 to 11 days (grade 1) after instillation; - animal #: 24 and 48 h (grade 3), 72 h (grade 2) and 4 to 1 5 days (grade 1) after instillation. The fluorescein test performed 24 hrs after instillation revealed corneal staining in all animals (whole surface). The fluorescein test performed 7 days after instillation revealed corneal staining in animal # and #3 (½ the surface) and animal #2 (¼ of the surface). The fluorescein test performed 14 days after instillation revealed corneal staining in animal nos. 1 and 3 (¼ of the surface). *Iritis* Irritation of the iris (grade 1) was observed in all animals 24 h to 7 days after instillation, in animal #1 until 19 days and in animal #3 until 8 days after instillation. *Conjunctival redness* - all animals (grade 1 to 3) observed 1 hour to 8 days after instillation. *Conjunctival chemosis* - all animals (grade 1 to 3) observed 1 to 72 h after instillation, in animal #2 until 5 days after instillation. In addition, whitish deposits (likely pus) was observed in animal #1 48 h to 5 days after instillation.

Table CP 7.1.5/01-1:Summary of eye irritation scores according to the Draize scheme: Individual and mean skin irritation

Time point	Cornea opacity			Iris (redness)			Conjunctival erythema			Conjunctival chemosis		
	Animal number											
	1	2	3	1	2	3	1	2	3	1	2	3
Spiroxamine 500 g/L EC applied at 1%												
1 h	0	0	0	0	0	1	1	1	1	1	1	1
1 h	0	0	0	0	0	0	1	1	1	1	1	1
24 h (day 1)	3	3	3	1	1	1	3[a]	3[a]	2[a]	2	2	3
48 h (day 2)	3	3	3	1	1	1	3[b]	2	1	1	2	2
72 h (day 3)	2	2	2	1	1	1	1[b]	2	1	1	1	1
4 days	2	2	1	1	1	1	1[b]	1	1	0	1	0
5 days	2	1	1	1	1	1	1[b]	1	1	0	1	0
6 days	2	1	1	1	1	1	1	1	1	0	0	0
7 days	2	1	1	1	1	1	1	1[d]	1	0	0	0
8 days	1	1	1	1	0	1	1	1	1	0	0	0
9 days	1	1	1	1	0	0	0	0	0	0	0	0
10 days	1	1	1	1	0	0	0	0	0	0	0	0
11 days	1	1	1	1	0	0	0	0	0	0	0	0
12 days	1	0	1	1	0	0	0	0	0	0	0	0
13 days	1	-	1	1	-	0	0	-	0	0	-	0
14 days	0	-	1	1	-	0	0[d]	-	0[d]	0	-	0
15 days	1	-	1	1	-	0	0	-	0	0	-	0
16 days	1	-	-	1	-	0	0	-	0	0	-	0
17 days	1	-	-	1	-	-	0	-	-	0	-	-
18 days	1	-	-	1	-	-	0	-	-	0	-	-
19 days	1	-	-	1	-	-	0	-	-	0	-	-
20 days	0	-	-	0	-	-	0	-	-	0	-	-
Mean (24 – 72 h)	2.7	2.7	2.7	1.0	1.0	1.0	2.3	2.3	1.3	1.3	1.7	2.0
	2.7			1.0			2.0			1.7		

- not examined
a corneal staining, whole surface
b pus in conjunctival sac
c corneal staining, ½ of the surface
d corneal staining, ¼ of the surface

C. Body weight and food consumption:

1. Body weight: Animals were only weighed at the beginning of the study, thus effects on body weight cannot be assessed.

2. Food consumption: Not applicable.

D. Necropsy:

Not undertaken.

E. Deficiencies:

Whilst it is recognised under the current guidance and the requirements of (EU) 284/2013 that a tiered testing strategy should be followed with a validated *in vitro* test method, this approach has not been adopted. However, the study was conducted prior to the publication of the EU commission regulation and validation of acceptable *in vitro* alternatives. These *in vivo* data are however considered valid to address this endpoint.

Assessment and conclusions by applicant:

Assessment: Study meets the current guidance and the requirements in 284/2013.

Conclusion: Under the conditions of this study the test article, Prothioconazole + Spiroxamine EC 460 showed irreversible eye damage. According to Annex I for Regulation (EC) 1272/2008, Prothioconazole + Spiroxamine EC 460 is classified as Eye Damage/Irritation, Category 2, H319 (causes serious eye irritation).

CP 7.1.6 Skin sensitization

Data Point:	KCP 7.1.6/01
Report Author:	[redacted]
Report Year:	2002
Report Title:	JAU 6476 160 EC & KWG 4168 300 - Study for the skin sensitization effect in guinea pigs (guinea pig maximization test according to Magnusson and Kligman)
Report No:	32072
Document No:	M-066247-01-1
Guideline(s) followed in study:	OECD 406; Guideline 96/54/EC, Method B.6.; US-EPA 712-C-98-197, OPPTS 870.2600
Deviations from current test guideline:	Yes Although the study was conducted according to test guideline OECD 406 (1922) the following deficiency is noted: The sensitivity and reliability of the experimental technique used should be assessed every 6 months by known positive controls (e.g. hexyl cinnamic aldehyde). Whilst the study report states that this has been undertaken, no details are provided (i.e. concentrations used, when conducted).
Previous evaluation:	yes, evaluated and accepted RAR (2010)
GLP/Officially recognised testing facilities:	Yes, conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	Yes

Executive Summary

A Magnusson and Kligman (M&K) Maximization assay was conducted in guinea pigs in order to examine the skin sensitisation potential of Prothioconazole + Spiroxamine EC 460. Following a preliminary test, the test article was dissolved in saline and initially administered *via* intradermal injection at 1% with and without Freund's complete adjuvant (FCA) to 20 animals. A corresponding control group (10 animals) received saline with and without FCA. A topical induction application was undertaken 1 week later with 2% Prothioconazole + Spiroxamine EC 460applied for 48 hours to test article treated animals. The control group was treated with saline only.

Topical challenge with 3% Prothioconazole + Spiroxamine EC 460 was undertaken 2 weeks post the topical induction. Skin reactions were recorded at 48 and 72 hours after the challenge applications with no erythema observed in either control or test article treated animals

Therefore, according to the evaluation criteria for the M&K assay with <30% animals exhibiting erythema, Prothioconazole + Spiroxamine EC 460 is considered not a skin sensitiser

Sensitivity and specificity of the test system was reported to be demonstrated at the conducting laboratory, however details of when this was performed and at what concentration of α-hexylcinnamaldehyde administered were not detailed.

Under the conditions of this study the Prothioconazole + Spiroxamine EC 460 was confirmed to not be a skin sensitiser when examined in the guinea pig employing the maximisation methodology. Therefore, according to Annex I for Regulation (EC) 1272/2008 the formulation has no obligatory labelling requirement for skin sensitisation and is unclassified.

Materials and Methods

A. Materials:

1. Test Material:	Prothioconazole + Spiroxamine EC 460 (alternative name: JAU 6476 160 EC & KWG 4168 300)
Description:	Clear dark-yellow liquid
Lot/Batch No.:	06920/0045(0019)
Purity:	157.44 g/L (prothioconazole); 300.12 g/L (spiroxamine)
CAS No.:	178928-70-6 (prothioconazole); 118134-30-8 (spiroxamine)
Stability of test compound:	Confirmed stable for the duration of the study (expiry date: 30 April 2002)
2. Vehicle and/or positive control:	Saline / not included but checked for sensitivity and specificity with α-hexylcinnamaldehyde
3. Test animals:	
Species:	Guinea pig
Strain:	Hsd Poc:DH
Age at dosing:	*ca.* 5 weeks
Weight at dosing:	248 - 354g
Source:	Winkelmann, Borchen, District of Paderborn
Acclimation period:	At least 5 days
Diet:	PROVIMI KLIBA 3420 diet, *ad libitum*
Water:	Municipal water, *ad libitum*
Housing:	Housed 5 animals/cage
4. Environmental conditions:	
Temperature:	22 ±3°C
Humidity:	50 ±10%
Air changes:	*ca.* 10/h
Photoperiod:	12 hour light/dark

B. Study Design:

1. In life dates:	19 February 2002 to 15 March 2002 (experimental dates)
2. Preliminary range finder:	A single guinea pig was injected intradermally, twice with 0.1 mL of Prothioconazole + Spiroxamine EC 460 at the following concentrations: 0, 1, 2.5 and 5%. The injection sites were assessed after 24 and 48 hours. For topical induction two dose range finding experiments were undertaken: - 4 guinea pigs had Prothioconazole + Spiroxamine EC 460 applied at 0, 25, 50 and 100%. Each animal was treated with 0.5 mL of test article soaked into a dressing and fixed under an occlusive dressing for 24 hours. At the end of the exposure period the test article was wash from the test site with saline. Skin reactions were assessed at 48 and 72 hours post application.

- 2 guinea pigs had Prothioconazole + Spiroxamine EC 460 applied at 0, 3, 6 and 12%. Each animal was treated with 0.5 mL of test article soaked into a dressing and fixed under an occlusive dressing for 24 hours. At the end of the exposure period the test article was wash from the test site with saline. Skin reactions were assessed at 48 and 72 hours post application.
- For challenge, 2 guinea pigs from the 2nd topical induction dose range finder had Prothioconazole + Spiroxamine EC 460 applied at 0, 1, 3 and 6%. Each animal was treated with 0.5 mL of test article soaked into a dressing and fixed under an occlusive dressing for 24 hours. At the end of the exposure period the test article was wash from the test site with saline. Skin reactions were assessed at 48 and 72 hours post application.

Based on these results from the range finder experiment concentrations selected for the main study were:

- Intradermal induction: 1%
- Topical induction: 12%
- Challenge: 3%

3. Animal assignment and treatment:

Forty albino guinea-pigs of the Hsd Poc:DH strain were allocated to two groups as follows and the Magnusson & Kligman method was used to determine the skin sensitisation potential of Spiroxamine:

1. Control group: 10 animals
2. Prothioconazole + Spiroxamine EC 460: 30 animals

Intradermal injections:

Test article group:

- Cranial/bilateral site: Freund's complete adjuvant (FCA) diluted 1:1 with saline
- Medial/bilateral site: Prothioconazole + Spiroxamine EC 460 1% formulated in saline
- Caudal/bilateral site: Prothioconazole + Spiroxamine EC 460 1% formulated in saline and FCA in equal parts.

Control groups:

- Treated as above, but with Prothioconazole + Spiroxamine EC 460 replaced with a corresponding volume of saline or saline and FCA for medial and caudal injection sites, respectively.

Topical induction (1 week later):

Treatment sites were clipped the day prior to application. Hypoallergenic dressings (2 x 4 cm) were applied on the injection sites covered with aluminium foil and fixed to the skin with adhesive tape. The dressing contained the following:

Test article group:

- 0.5 mL Prothioconazole + Spiroxamine EC 460, 12%

Control groups:

- Treated as above, but with 0.5 mL of saline

At the end of the exposure period (48 hours), test article was removed with saline solution.

Topical challenge (3 weeks after intradermal injections):

Treatment sites were clipped the day prior to application. Hypoallergenic dressings (2 x 4 cm) were applied on the injection sites covered with aluminium foil and fixed to the skin with adhesive tape. The dressings contained the following for both control and test article treated animals:

Challenge

- 0.5 mL of Prothioconazole + Spiroxamine EC 460 (3%) were fixed on to the right caudal positions)
- A dressing soaked in saline solution was placed on the right flank to served as a control.

At the end of the exposure period (24 hours), test article was removed with saline solution.

	Skin reactions were recorded at 48 and 72 hours after the challenge applications.	
4. Evaluation criteria:	No visible change:	0
	Discrete or patch erythema	1
	Moderate and confluent erythema	2
	Intense erythema and swelling	3
5. Interpretation criteria:	Evidence of skin sensitization potential was evaluated against the following criteria: - Redness (score ≥ 1) in 30% of the test animals using the adjuvant test	
5. Statistics:	Not undertaken	

C. Methods:

1. Homogeneity and achieved concentration analysis of the dose:	None.
2. Observations:	Animals were observed daily for clinical signs of toxicity throughout the experimental period. The application sites were observed at the end of exposure period, with skin reactions recorded at 48 and 72 hours after the challenge applications.
3. Body weights:	Animals were weighed prior to study start and on day 24.
4. Food consumption:	Not recorded.
5. Sacrifice and pathology:	Not undertaken

Results and Discussion:

A. Homogeneity and achieved concentration analysis:

Not undertaken. Analyses for achieved concentration, homogeneity or stability of test article formulations were not conducted as part of this study, as this is not a requirement of the regulatory test guidelines.

B. Preliminary range finder experiment:

1. Intradermal induction:

Following intradermal injections twice at 0.1, 2.5 and 5% the following observations were noted at 24 and 48 h:

0%:
- no reaction

1%:
- white wheal with red surrounding areas at 24 h. At 48 h the white wheal had turned to grey wheal, with the redness remaining.

2.5 and 5%:
- great grey wheal (swollen mark), with greater red surrounding area after 24 h. At 48 h the grey wheal had turned to black wheal, with the redness remaining.

2. Topical induction:

Following topical induction at 0,12, 25, 50, 100% the following observations were noted at 48 and 72 h:

0, 3, 6%:
- no erythema at 48 or 72 h

12%:
- 1/2 animals no erythema at 48 or 72 h
- 1/2 animals displayed grade 1 erythema at 48 and 72 h

25%:
- all four animals displayed grade 1 erythema at 48 and 72 h

50%:
- 3/4 animals displayed grade 2 erythema at 48 and 72 h.
- A single animal displayed grade 3 erythema at 48 and 72 h.

100%:
- 1/4 animals displayed grade 2 erythema at 48 and 72 h
- 1/4 animals displayed grade 3 erythema at 48 h and grade 2 erythema at 72 h
- 1/4 animals displayed grade 2 erythema at 48 h and grade 1 erythema at 72 h
- 1/4 animals displayed grade 1 erythema at 48 h and grade 2 erythema at 72 h

3. Challenge: Following challenge application at 0,, 1, 3, 6% the following observations were noted at 48 and 72 h:
0, 1, 3%:
- no erythema at 48 or 72 h

6%:
- 2/2 animals displayed grade 1 erythema at 48 h and no erythema at 72 h

C. Observations:

1. Clinical signs of toxicity: No clinical signs of toxicity were observed in either control groups or the test article treated animals.

2. Mortality: No test article-related deaths were observed.

3. Skin reactions: After the intradermal induction the animals in the control group showed red wheal at the application site at 48 h.
Test article treated animals showed red/white wheal with or without red surrounding area, red injection site encrustation at day 7 wheals and encrustations were observed.
Following challenge with 3% Prothioconazole + Spiroxamine EC 460 at 48 and 72 hours no erythema was observed in either control or test article treated animals.
Sensitivity and specificity of the test system was reported to be demonstrated at the conducting laboratory, however details of when this was performed and at what concentration of α-hexylcinnamaldehyde administered were not detailed.

Table CP 7.1.6/01-1: Overview of skin sensitisation study in guinea pigs treated with Prothioconazole + Spiroxamine EC 460: scores according to the Magnusson and Kligman grading

Conc. (%)	Control patch		Test article patch	
	48 h	72 h	48 h	72 h
3%	0/10	0/10	0/20	0/20
Total	1/10		0/20	

no. of animals with skin reddening/total no. of animals treated

D. Body weight and food consumption:

1. Body weight: All animals gained weight during the dosing and observation period

2. Food consumption: Not applicable

Table CP 7.1.6/01-2: Overview of skin sensitisation study in guinea pigs treated with Prothioconazole + Spiroxamine EC 460: body weight

Day	Control group	Test article
0	305 ±41.6	312 ±22.3
24	458 ±47.8	490 ±30.3
Net body weight gain (g)	153 ±39.1	178 ±26.9

E. Necropsy:

Not conducted

F. Deficiencies:

Although the study was conducted according to test guideline OECD 406 (1922) the following deficiency is noted:

- The sensitivity and reliability of the experimental technique used should be assessed every 6 months by known positive controls (*e.g.* hexyl cinnamic aldehyde). Whilst the study report states that this has been undertaken, no details are provided (*i.e.* concentrations used, when conducted).

In conclusion, the data generated under this study are considered valid.

Whilst it is recognised under the current guidance and the requirements of (EU) 283/2013 that the preferred test method to assess skin sensitisation is the LLNA assay this has not been condiction in this case. However, the study was conducted prior to the publication of the EU commission regulation and validation of the LLNA. Where a guinea pig assay (maximisation assay) is available, demonstrating a clear result, further testing is not deemed warranted for animal welfare reasons. These *in vivo* data are considered valid to address this endpoint.

Assessment and conclusion by applicant:

Assessment: This study is deemed acceptable and meets the requirements in 283/2013.

Conclusion: Under the conditions of this study the Prothioconazole + Spiroxamine EC 460 was confirmed to not be a skin sensitiser when examined in the guinea pig employing the maximisation methodology. Therefore, according to Annex I for Regulation (EC) 1272/2008 the formulation has no obligatory labelling requirement for skin sensitisation and is unclassified.

CP 7.1.7 Supplementary studies on the plant protection product

No such studies are necessary since there are no concerns arising, e.g., from potential synergistic or additive effects exerted by the active substance(s) or other components in Prothioconazole + Spiroxamine EC 460 (160+300 g/L) that would require further investigations.

CP 7.1.8 Supplementary studies for combinations of plant protection products

No such studies are necessary since Prothioconazole + Spiroxamine EC 460 (160+300 g/L) is not intended for use in combination with other plant protection products.

CP 7.2 Data on exposure

Evaluations of the exposure of operators, bystanders, residents and re-entry workers to prothioconazole, prothioconazole-desthio and spiroxamine when used in the Prothioconazole + Spiroxamine EC 460 (160+300 g/L) formulation are provided in the following sections. The relevant representative uses for assessment of exposure are shown in Table CP 7.2-1.

Table CP 7.2-1 Representative uses of Prothioconazole + Spiroxamine EC 460 (160+300 g/L) for exposure assessment

Crop (field / indoor)	No. of applications (interval)	Application rate (kg a.s/ha)	Water volume L/ha	Application equipment
Barley, oats (field) [BBCH 30-61]	1 – 2[a] (14-21d interval)	[0.50 – 1.25 L/product] 0.08 - 0.2 (PTZ) 0.15 - 0.375 (SPX)	100[b] – 400	Tractor-mounted conventional boom sprayer

Crop (field / indoor)	No. of applications (interval)	Application rate (kg a.s/ha)	Water volume L/ha	Application equipment
Wheat, rye, triticale (field) [BBCH 30-69]	1 – 2[a] (14-21d interval)	[0.50 – 1.25 L/product] 0.08 - 0.2 (PTZ) 0.15 - 0.375 (SPX)	100[b] – 400	Tractor-mounted conventional boom sprayer

a. maximum number of applications per year
b. Produces the highest spray concentration

The formulation will be applied to the representative crops in the EU by professionals using tractor-mounted conventional boom sprayers for cereals outdoors.

Outdoor exposure estimates have been calculated using the EFSA model (updated model released 30 March 2015):

> *EFSA (European Food Safety Authority), 2014. Guidance on the assessment of exposure of operators, workers, residents and bystanders in risk assessment for plant protection products. EFSA Journal 2014;12(10):3874, 55 pp.*

This guidance document was adopted in the Standing Committee on Plants, Animals, Food and Feed on 29 May 2015 and will apply to applications submitted from 1 January 2016. The Standing Committee on Plants, Animals, Food and Feed agreed on 24 January 2017 to revise the implementation schedule for this guidance with the consideration of acute exposure assessments where an AAOEL has been established *i.e.* acute operator, worker and bystander exposure assessments can be performed where an AAOEL (acute acceptable operator exposure level, termed RVAAS [Reference Value Acutely toxic Active Substance] in the EFSA model) has been established. The AAOEL is typically derived from the ARfD, with oral absorption correction made where required. An AAOEL has been proposed for spiroxamine, and therefore this reference value has been used to quantify the acute risk to operators, workers and bystanders. Details for AOEL and AAOEL values for prothioconazole and prothioconazole-desthio used for the non-dietary risk assessment are detailed in Table CA CP 7.3-01.

The input parameters for the EFSA model calculations are detailed in the relevant sections.

The default body weight using the EFSA model is 60 kg. Dermal absorption values are shown in Table CP 7.3-01.

The product is a mixture of two active substances.

From a scientific point of view it is regarded necessary to take into account potential combination effects. However, the evaluation of cumulative or synergistic effects as requested by Art. 4 (3b) of Regulation (EC) No. 1107/2009 should only be performed when harmonised “scientific methods accepted by the Authority to assess such effects are available.” In the absence of a harmonised approach, this assessment has not been undertaken.

CP 7.2.1 Operator exposure

The application of Prothioconazole + Spiroxamine EC 460 (160+300 g/L) to cereals at the maximum application rate and at a minimum spray volume, as indicated in Table CP 7.2-1 represents the worst case potential exposure to operators.

The Operator Outdoor Spray AOEM (within the EFSA model) was used to estimate exposures. Dermal absorption values used to estimate systemic exposure to prothioconazole (prothioconazole-desthio) and spiroxamine for the concentrate and spray dilutions for Prothioconazole + Spiroxamine EC 460 (160+300 g/L) are detailed in Table CP 7.2.1.1-2.

A summary of the estimated exposure of operators to spiroxamine as a result of the critical exposure scenarios with and without the use of PPE are shown in Table CP 7.2.1-1.

Table CP 7.2.1-1 Summary of estimations of operator exposure in relation to the AOEL and AAOEL from refinement with operator exposure study

Parameters	Level of PPE	Total absorbed dose (mg/kg bw/d)		%AOEL	%AAOEL	Reference
		Long term	Short term			
Tractor-mounted boom sprayer application outdoors to cereals *Application rate: 1.25 L product/ha*						
• 0.2 kg PTZ/ha • 60 kg[1]	Protective garment[2,3]	0.00103	-	0.52	-	Table CP 7.2.1.2-1 (exposure estimate)
• 0.2 kg PTZ desthio/ha • 60 kg[1]	Protective garment[2,3]	0.00108		10.8	-	Table CP 7.2.1.2-2 (exposure estimate)
• 0.375 kg SPX/ha • 60 kg[1]	Protective garment[2,3]	0.00147	0.00147	9.8	2.41	Table CP 7.2.1.2-3 (exposure estimate)

1 Default body weight for EFSA model
2 Protective garment defined as operator wearing a work wear clothing covering arms, body and legs
3 Closed cabin used in study

Conclusion

The algorithms used to estimate operator exposures are embedded in the model and use data from the 75th percentile.

According to the EFSA model calculations it can be concluded that the risk for operators exposed to the active ingredients, prothioconazole (its metabolite, prothioconazole-desthio) and spiroxamine in Prothioconazole + Spiroxamine EC 460 (160+300 g/L) is acceptable following application to field (low) crops PPE in the form of visor, hood and gloves are worn during mixing/loading and application.

Operator exposure studies conducted to estimate exposure to the active ingredients, prothioconazole (its metabolite, prothioconazole desthio) and spiroxamine (when prothioconazole equivalents are used) when normal work wear and use of a closed cabin are considered confirms acceptable exposure to the active ingredient prothioconazole and its metabolite prothioconazole-desthio with long term exposure between <11% of the AOEL and short and long-term exposure to spiroxamine of 9.8% and 2.41% of the AOEL and AAOEL, respectively.

Therefore is can be concluded that the risk for operators exposed to the active ingredients in Prothioconazole + Spiroxamine EC 460 (160+300 g/L) is acceptable following application to field (low) crops. Due to the classification of the formulation (Skin Corrosion/Irritation, Cat. 2, H315; Eye Damage/Irritation, Cat. 2, H319; PPE in the form of gloves and hood to protect eyes and skin is recommended during mixing/loading and application. Drift technology is required to address resident/bystander exposure.

CP 7.2.1.1 Estimation of operator exposure

The Operator Outdoor Spray AOEM in the EFSA guidance was used to estimate exposures for operators applying Prothioconazole + Spiroxamine EC 460 (160+300 g/L) to cereals. The EFSA glasshouse model was used to estimate operator exposure following outdoor application.

The following parameters and assumptions have been used in calculating operator exposure.

Table CP 7.2.1.1-1 Application data for operators

Crop scenario	Area treated/day	Application rate
Low outdoor crops (cereals)	50 ha/day (default for tractor-mounted boom sprayer)	0.5 – 1.25 L/product/ha

Table CP 7.2.1.1-2 Penetration and absorption data

Category of absorption	Penetration/absorption rate		
Standard protective garment (work wear covering, arms, body and legs) during handling of the concentrate or application of the diluted product	10% [General/default value for all formulations (EFSA, 2015)]		
Hood and visor (dermal exposure – head only)	5% [General/default value for all formulations (EFSA, 2015)]		
Absorption of oral material	61% [Refer to MCA Section 5]		
Absorption of inhaled material	100% [In absence of specific data (EFSA, 2015)]		
	PTZ	**PTZ desthio**	**SPX**
Dermal absorption through exposure to the concentrate (mixing/loading)	25% (default value) [EFSA Journal 2017; 15(6):4873]		0.87% (300 g/L) [CP 7.3/02]
Dermal absorption through exposure to the spray dilution	47% (0.26 g/L [1:1.615 dilution]) [CP 7.3/02, M-758748-01-1]		22% (0.9375 g/L [1:320 dilution]) [CP 7.3/01, M-758748-01-1]
AOEL	0.2 mg/kg bw/d[a] [EFSA Scientific Report (2007), 106, 1-98]	0.01 mg/kg bw/d[b] [EFSA Scientific Report (2007), 106, 1-98]	0.015 mg/kg bw/d[e] [Refer to MCA Section 5]
AAOEL	-	-	0.061 mg/kg bw[e] [Refer to MCA Section 5]

PTZ [Prothioconazole SANCO/3923/07 final 26 January 2021]

a. 0.2 mg/kg bw/d (based on NOAEL in the rat developmental study conducted with PTZ, with a 100-fold assessment factor, no correction for oral absorption required (ADME data in the rat indicate oral absorption [>90%]))

PTZ desthio [may be formed in diluted PTZ formulations, particularly on cloning, skin and plant surfaces during drying process].

c. 0.01 mg/kg bw/d (based on NOAEL in the rat developmental study conducted with PTZ desthio, with a 100-fold assessment factor, no correction for oral absorption required (ADME data in the rat indicate oral absorption [>90%]))

SPX:

d. 0.015 mg/kg bw/d (based on the NOAEL in the dog 1-year dietary study, with an application of a 100-fold assessment factor, correction for oral absorption required (ADME data in the rat indicate oral absorption [61%]))
e. 0.061 mg/kg bw (based on the NOAEL in the rat, acute neurotoxicity study with an application of a 100-fold assessment factor, correction for oral absorption required [61%]))

Standard methodology for determining the potential exposure to operators requires that a tiered approach be adopted, whereby a 'Tier I' assessment is conducted in which it is assumed that no personal protective equipment (PPE) is used. The estimated exposures were compared with the AOELs of 0.25, 0.2 and 0.015 mg/kg bw/day and AAOELs of 0.08, 0.01 and 0.061 mg/kg bw, for long and short term system exposure for PTZ, PTZ desthio and SPX, respectively. The default body weight for an operator is 60 kg using the EFSA model.

The algorithms used to estimate operator exposures are embedded in the EFSA model and use data from the 75th percentile. The input parameters used to estimate operator exposure are presented in Table CP 7.2-2. The outputs of the EFSA model are presented in Table CP 7.2.1.1-3 and Table CP 7.2.1.1-4 (Note: RVNAS and RVAAS are the same as the AOEL and AAOEL, respectively).

Table CP 7.2.1.1-3 Summary of estimations of operator exposure in relation to the AOEL and AAOEL

Model data	Level of PPE	Total absorbed dose (mg/kg bw/d)		%AOEL	%AAOEL	Reference
		Long term	Short term			
Tractor-mounted boom sprayer application outdoors to cereals *Application rate: 1.25 L product/ha*						
EFSA model • 0.2 kg PTZ/ha • 50 ha/day[1] • 2-3 m buffer[1] • 60 kg[3]	No PPE[2]	0.2149	-	107.44	-	Table CP 7.2.1.1-4 (input parameter) Table CP 7.2.1.1-5 (exposure estimate)
	Protective garment[4]	0.1343	-	67.16	-	
	PPE[5]	0.0030	-	1.48	-	Table CP 7.2.1.1-6 (exposure estimate)
EFSA model • 0.2 kg PTZ desthio/ha • 50 ha/day[1] • 2-3 m buffer[1] • 60 kg[3]	No PPE[2]	0.2149	-	2148.77	-	Table CP 7.2.1.1-7 (input parameter) Table CP 7.2.1.1-8 (exposure estimate)
	Protective garment[4]	0.1343	-	1343.24	-	
	PPE[5]	0.0016	-	15.64	-	Table CP 7.2.1.1-9 (exposure estimate)
EFSA model • 0.375 kg SPX/ha • 50 ha/day[1] • 2-3 m buffer[1] • 60 kg[3]	No PPE[2]	0.0277	0.1552	184.63	254.37	Table CP 7.2.1.1-10 (input parameter) Table CP 7.2.1.1-11 (exposure estimate)
	Protective garment[4]	0.0180	0.1013	119.98	165.99	
	PPE[5]	0.0012	0.0194	8.27	31.83	Table CP 7.2.1.1-12 (exposure estimate)

Table CP 7.1.1.2-4: Input parameters for the EFSA model for the active substance prothioconazole when applied to cereals (field), tier I assessment for operators, bystanders, residents and worker exposure

Substance name	Prothioconazole (PTZ)	
Product name	PTZ + SPX EC 460 (160+300 g/L)	
Reference value non acutely toxic active substance (RVNAS)	0.2	mg/kg bw/day
Reference value acutely toxic active substance (RVAAS)		mg/kg bw/day
Crop type	Cereals	
Substance properties		
Formulation type	Soluble concentrates, emulsifiable concentrate, etc.	
Miniumum volume water for application (liquids)	100	L/ha
Maximum application rate of active substance	0.3	kg a.s./ha
50% Dissipation Time DT_{50}	30	days
Initial Dislodgeable Foliar Residue	3	$\mu g/cm^2$ of foliage/kg a.s. applied/ha
Dermal absorption of product	25.00%	
Dermal absorption of in-use dilution	47.00%	
Oral absorption of active substance	100.00%	
Inhalation absorption of active substance	100.00%	
Vapour pressure of active substance	low volatile substances having a vapour pressure of <5*10-3Pa	
Scenario		
Indoor or Outdoor application	Outdoor	
Application method	Downward spraying	
Application equipment	Vehicle-mounted	
Buffer strip	10	m
Number of applications	2	
Interval between multiple applications	14	days
Season (upward spraying orchards only)	not relevant	

Table CP 7.2.1.1-5 Operator outdoor spray AOEM results for field application of Prothioconazole + Spiroxamine EC 460 (160+300 g/L) to cereals (0.2 kg PTZ/ha) without PPE – tractor-mounted boom sprayer application

Operator Model		Mixing, loading and application AOEM			
Potential exposure	Longer term systemic exposure (mg/kg bw/day)		0.2149	% of RVNAS	107.44%
	Acute systemic exposure (mg/kg bw/day)		1.1752	% of RVAAS	
Mixing and Loading	Gloves = No	Clothing = Work wear - arms, body and legs covered		RPE = None	Soluble bags = No
Application	Gloves = No	Clothing = Work wear - arms, body and legs covered		RPE = None	Closed cabin = No
Exposure (including PPE options above)	Longer term systemic exposure (mg/kg bw/day)		0.1343	% of RVNAS	67.16%
	Acute systemic exposure (mg/kg bw/day)		0.5624	% of RVAAS	

Table CP 7.2.1.1-6 Operator outdoor spray AOEM results for field application of Prothioconazole + Spiroxamine EC 460 (160+300 g/L) to cereals (0.2 kg PTZ/ha) with PPE – tractor-mounted boom sprayer application

Operator Model		Mixing, loading and application AOEM			
Potential exposure	Longer term systemic exposure (mg/kg bw/day)		0.2149	% of RVNAS	107.44%
	Acute systemic exposure (mg/kg bw/day)		1.1752	% of RVAAS	
Mixing and Loading	Gloves = Yes	Clothing = Work wear - arms, body and legs covered		RPE = Hood and visor	Soluble bags = No
Application	Gloves = Yes	Clothing = Work wear - arms, body and legs covered		RPE = Hood and visor	Closed cabin = No
Exposure (including PPE options above)	Longer term systemic exposure (mg/kg bw/day)		0.0030	% of RVNAS	1.48%
	Acute systemic exposure (mg/kg bw/day)		0.0503	% of RVAAS	

Table CP 7.1.1.2-7: Input parameters for the EFSA model for the metabolite prothioconazole desthio when applied to cereals (field), tier I assessment for operators, bystanders, residents and worker exposure

Substance name	Prothioconazole desthio (PTZ desthio)	
Product name	PTZ + SPX EC 460 (160+300 g/L)	
Reference value non acutely toxic active substance (RVNAS)	0.01	mg/kg bw/day
Reference value acutely toxic active substance (RVAAS)		mg/kg bw/day
Crop type	Cereals	
Substance properties		
Formulation type	Soluble concentrates, emulsifiable concentrate, etc.	
Miniumum volume water for application (liquids)	100	L/ha
Maximum application rate of active substance	0.3	kg a.s./ha
50% Dissipation Time DT_{50}	30	days
Initial Dislodgeable Foliar Residue	3	$\mu g/cm^2$ of foliage/kg a.s. applied/ha
Dermal absorption of product	25.00%	
Dermal absorption of in-use dilution	47.00%	
Oral absorption of active substance	100.00%	
Inhalation absorption of active substance	100.00%	
Vapour pressure of active substance	low volatile substances having a vapour pressure of <5*10-3Pa	
Scenario		
Indoor or Outdoor application	Outdoor	
Application method	Downward spraying	
Application equipment	Vehicle-mounted	
Buffer strip	2-3	m
Number of applications	2	
Interval between multiple applications	14	days
Season (upward spraying orchards only)	not relevant	

Table CP 7.2.1.1-8 Operator outdoor spray AOEM results for field application of Prothioconazole + Spiroxamine EC 460 (160+300 g/L) to cereals (0.2 kg PTZ desthio/ha) without PPE – tractor-mounted boom sprayer application

Operator Model		Mixing, loading and application AOEM		
Potential exposure	Longer term systemic exposure (mg/kg bw/day)	0.2149	% of RVNAS	2148.77%
	Acute systemic exposure (mg/kg bw/day)	1.1752	% of RVAAS	
Mixing and Loading	Gloves = No	Clothing = Work wear - arms, body and legs covered	RPE = None	Soluble bags = No
Application	Gloves = No	Clothing = Work wear - arms, body and legs covered	RPE = None	Closed cabin = No
Exposure (including PPE options above)	Longer term systemic exposure (mg/kg bw/day)	0.1343	% of RVNAS	1343.24%
	Acute systemic exposure (mg/kg bw/day)	0.5624	% of RVAAS	

Table CP 7.2.1.1-9 Operator outdoor spray AOEM results for field application of Prothioconazole + Spiroxamine EC 460 (160+300 g/L) to cereals (0.2 kg PTZ desthio/ha) with PPE – tractor-mounted boom sprayer application

Operator Model		Mixing, loading and application AOEM		
Potential exposure	Longer term systemic exposure (mg/kg bw/day)	0.2149	% of RVNAS	2148.77%
	Acute systemic exposure (mg/kg bw/day)	1.1752	% of RVAAS	
Mixing and Loading	Gloves = Yes	Clothing = Work wear - arms, body and legs covered	RPE = Hood and visor	Soluble bags = Yes
Application	Gloves = Yes	Clothing = Work wear - arms, body and legs covered	RPE = Hood and visor	Closed cabin = Yes
Exposure (including PPE options above)	Longer term systemic exposure (mg/kg bw/day)	0.0016	% of RVNAS	15.64%
	Acute systemic exposure (mg/kg bw/day)	0.0364	% of RVAAS	

Table CP 7.1.1.1-10 Input parameters for the EFSA model for the active substance spiroxamine when applied to cereals (field), tier I assessment for operators, bystanders, residents and worker exposure

Substance name	Spiroxamine (SPX)	
Product name	PTZ + SPX EC 460 (160+300 g/L)	
Reference value non acutely toxic active substance (RVNAS)	0.015	mg/kg bw/day
Reference value acutely toxic active substance (RVAAS)	0.061	mg/kg bw/day
Crop type	Cereals	
Substance properties		
Formulation type	Soluble concentrates, emulsifiable concentrate, etc.	
Miniumum volume water for application (liquids)	100	L/ha
Maximum application rate of active substance	0.375	kg a.s./ha
50% Dissipation Time DT_{50}	30	days
Initial Dislodgeable Foliar Residue	3	$\mu g/cm^2$ of foliage/kg a.s. applied/ha
Dermal absorption of product	0.87%	
Dermal absorption of in-use dilution	22.00%	
Oral absorption of active substance	61.00%	
Inhalation absorption of active substance	100.00%	
Vapour pressure of active substance	low volatile substances having a vapour pressure of <5*10-3Pa	
Scenario		
Indoor or Outdoor application	Outdoor	
Application method	Downward spraying	
Application equipment	Vehicle-mounted	
Buffer strip	2-3	m
Number of applications	2	
Interval between multiple applications	14	days
Season (upward spraying orchards only)	not relevant	

Table CP 7.2.1.1-11 Operator outdoor spray AOEM results for field application of Prothioconazole + Spiroxamine EC 460 (160+300 g/L) to cereals (0.375 kg SPX/ha) without PPE – tractor-mounted boom sprayer application

Operator Model		Mixing, loading and application AOEM		
Potential exposure	Longer term systemic exposure (mg/kg bw/day)	0.0277	% of RVNAS	184.63%
	Acute systemic exposure (mg/kg bw/day)	0.1552	% of RVAAS	254.37%
Mixing and Loading	Gloves = No	Clothing = Work wear - arms, body and legs covered	RPE = None	Soluble bags = No
Application	Gloves = No	Clothing = Work wear - arms, body and legs covered	RPE = None	Closed cabin = No
Exposure (including PPE options above)	Longer term systemic exposure (mg/kg bw/day)	0.0180	% of RVNAS	119.98%
	Acute systemic exposure (mg/kg bw/day)	0.1013	% of RVAAS	165.99%

Table CP 7.2.1.1-12 Operator outdoor spray AOEM results for field application of Prothioconazole + Spiroxamine EC 460 (160+300 g/L) to cereals (0.375 kg SPX/ha) with PPE – tractor-mounted boom sprayer application

Operator Model		Mixing, loading and application AOEM		
Potential exposure	Longer term systemic exposure (mg/kg bw/day)	0.0277	% of RVNAS	184.63%
	Acute systemic exposure (mg/kg bw/day)	0.1552	% of RVAAS	254.37%
Mixing and Loading	Gloves = Yes	Clothing = Work wear - arms, body and legs covered	RPE = Hood and visor	Soluble bags = No
Application	Gloves = Yes	Clothing = Work wear - arms, body and legs covered	RPE = Hood and visor	Closed cabin = No
Exposure (including PPE options above)	Longer term systemic exposure (mg/kg bw/day)	0.0012	% of RVNAS	8.27%
	Acute systemic exposure (mg/kg bw/day)	0.0194	% of RVAAS	31.83%

Conclusion

The algorithms used to estimate operator exposures are embedded in the model and use data from the 75th percentile.

According to the EFSA model calculations it can be concluded that the risk for operators exposed to the active ingredients, prothioconazole (its metabolite, prothioconazole-desthio) and spiroxamine in Prothioconazole + Spiroxamine EC 460 (160+300 g/L) is acceptable following application to field (low) crops PPE in the form of visor, hood and gloves are worn during mixing/loading and application.

Operator exposure studies conducted to estimate exposure to the active ingredients, prothioconazole (its metabolite, prothioconazole desthio) and spiroxamine (when prothioconazole equivalents are used) when normal work wear and use of a closed cabin are considered confirms acceptable exposure to the

active ingredient prothioconazole and its metabolite prothioconazole-desthio with long term exposure between <11% of the AOEL and short and long-term exposure to spiroxamine of 9.8% and 2.41% of the AOEL and AAOEL, respectively.

Therefore is can be concluded that the risk for operators exposed to the active ingredients in Prothioconazole + Spiroxamine EC 460 (160+300 g/L) is acceptable following application to field (low) crops. Due to the classification of the formulation (Skin Corrosion/Irritation, Cat. 2, H315; Eye Damage/Irritation, Cat. 2, H319; PPE in the form of gloves and hood to protect eyes and skin is recommended during mixing/loading and application. Drift technology is required to address resident/bystander exposure.

CP 7.2.1.2 Measurement of operator exposure

Collectively, estimated operator exposure to prothioconazole as well as to the metabolite, prothioconazole-desthio, and spiroxamine can be estimated using data from the mixing/loading and application specific exposure studies. The results of these studies are considered to represent higher tier data for the given application scenario than the EFSA model. These data have been used to model exposure to prothioconazole, prothioconazole-desthio and spiroxamine following application of Prothioconazole + Spiroxamine EC460.

Taking this approach, it can be concluded that operator exposure to prothioconazole, prothioconazole-desthio and spiroxamine in the formulation Prothioconazole + Spiroxamine EC 460 are below the respective AOEL and AAOEL assigned to each active.

Thus, Prothioconazole + Spiroxamine EC 460 (160+300 g/L) can be used in a manner consistent with label recommendations without potential risks to operators. Due to the classification of the formulation (Skin Corrosion/Irritation, Cat. 2, H315; Eye Damage/Irritation, Cat. 2, H319; PPE in the form of gloves and hood to protect eyes and skin is recommended during mixing/loading and application. As the exposure studies were conducted with tractors equipped with cabins (which is now the standard practice), this should be considered.

Table CP 7.2.1.2-1: Calculation of operator exposure to prothioconazole (0.20 kg PTZ/ha) for field application to cereals with PPE and closed cabin – tractor-mounted boom sprayer

Amount handled/day = treated area (ha/day) *x* use rate (kg PTZ/ha) = 50 *x* 0.20 = 10 kg PTZ/day						
Route of exposure	**Specific exposure (mg/kg PTZ)**		**Amount of active handled (kg PTZ/day)**	**Dermal absorption (%)**		**Estimated route specific exposure (mg/person/day)**
Dermal	0.012	*x*	10		=	0.12
Inhalation	0.00053	*x*	10		=	0.0053
Conversion of route specific exposure estimates to systemic exposure						
Dermal	0.12	*x*		47	=	0.0564
Inhalation	0.0053	*x*		100	=	0.0053
				Total	=	0.0617
				Total (mg/kg bw/day)	=	0.00103
				%AOEL (0.2 mg/kg bw/day)	=	0.52

Dermal = actual body + actual hand exposure
Inhalation = mixing/loading + application

Table CP 7.2.1.2-2: Calculation of operator exposure to prothioconazole-desthio (0.20 kg PTZ/ha) for field application to cereals with PPE and closed cabin – tractor-mounted boom sprayer

Amount handled/day = treated area (ha/day) *x* use rate (kg PTZ-desthio/ha) = 50 *x* 0.20 = 10 kg PTZ-desthio/day				
Route of exposure	**Specific exposure**	**Amount of active handled**	**Dermal absorption**	**Estimated route specific exposure**

	(mg/kg PTZ-desthio)		(kg PTZ desthio/day)	(%)		(mg/person/day)
Dermal actual	0.013	x	10		=	0.13
Inhalation [m/a]	0.00034	x	10		=	0.0034
Conversion of route specific exposure estimates to systemic exposure						
Dermal actual	0.13	x		47	=	0.0611
Inhalation [m/a]	0.0034	x		100	=	0.0034
			Total		=	0.0645
			Total (mg/kg bw/day)		=	0.00108
			%AOEL (0.01 mg/kg bw/day)		=	10.8

Table CP 7.2.1.2-3: Calculation of operator exposure to spiroxamine (0.375 kg SPX/ha) for field application to cereals with PPE and closed cabin – tractor-mounted boom sprayer

Amount handled/day = treated area (ha/day) x use rate (kg SPX/ha) = 50 x 0.375 = 18.75 kg SPX/day						
Route of exposure	**Specific exposure (mg/kg SPX)**		**Amount of active handled (kg SPX/day)**	**Dermal absorption (%)**		**Estimated route specific exposure (mg/person/day)**
Dermal	0.017	x	18.75	-	=	0.319
Inhalation	0.00096	x	18.75	-	=	0.018
Conversion of route specific exposure estimates to systemic exposure						
Dermal	0.319	x	-	22	=	0.0702
Inhalation	0.018	x	-	100	=	0.018
			Total		=	0.0882
			Total (mg/kg bw/day)		=	0.00147
			%AOEL (0.015 mg/kg bw/day)		=	9.8
			%AAOEL (0.061 mg/kg bw/day)		=	2.41

Dermal = actual body + actual hand exposure
Inhalation = mixing/loading + application

Measurement of operator exposure to prothioconazole and prothioconazole-desthio

The study reviews presented below are updated from the previous data presented by the RMS-DE -DE in the RAR Vol 3 B6 (2010) and where still relevant these positions have been included in the discussions below in this section.

Data Point:	KCP 7.2.1.2/01
Report Author:	[redacted]
Report Year:	2002
Report Title:	Determination of exposure to JAU 6476 and JAU 6476-desthio (SXX 0665) during mixing/loading and application of JAU 6476 in cereals
Report No:	MR-036/02
Document No:	M-040604-01-1
Guideline(s) followed in study:	OECD guidance document for the conduct of studies of occupational exposure to pesticides during agricultural application, Series on Testing and Assessment No. 9, 1997
Deviations from current test guideline:	None
Previous evaluation:	yes, evaluated and accepted RAR (2010)
GLP/Officially recognised testing facilities:	Yes, conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	Yes

Executive summary

An operator exposure study was carried out to measure the exposure of operators to prothioconazole and to its degradation product prothioconazole-desthio when applying Prothioconazole 250 EC in cereals, with the study designed as a mixer/loader/application exposure assessment. The applications were performed during the actual season on a field in Monheim, Germany. A total of eight monitorings at three different spray timings involving three different male operators were performed. With each monitoring about 20 ha were treated with tractor drawn/mounted ground boom sprayer. During the first two spray timings an equipment for larger field sizes was used (28 m boom, 2500 L water tank volume) whereas during the third spray timing equipment designed for smaller field sizes was chosen (15 m boom, 800 L water tank volume). The tractors used were all equipped with a cabin.

Dermal exposure was measured by passive dosimetry techniques: beneath usual work clothing (shirt and trousers) the operators wore cotton underwear, all clothing was used as sampling clothing. Exposure of the head was measured by a cap, exposure of the hands by hand washes (with detergent) and at the third spray timing in addition with isopropanol. Protective gloves worn during mixing/loading were rinsed at the end of the monitoring with acetonitrile.

Inhalation exposure was determined by use of a personal air sampling pump connected to an IOM-sampler with glass fibre filter, located in the breathing zone of the operator.

The spraying event lasted between 2.5 hours and 3.5 hours. On completion of the spraying the cap and the gloves were sampled and also a hand wash was performed. The operators continued to wear the other dosimeter clothes for some further hours to give a total of about 7 hours (one exception: *ca*. 5.2 hours) to provide some information on the proportion of conversion of prothioconazole to prothioconazole-desthio during the time of almost a full work day.

Samples were extracted, followed by LC-MS/MS determination. The results of the measurements are reported as determined (*i.e.*, µg a.s./sample) and as specific (normalized) exposures, *i.e.*, as mg of exposure/kg of a.s. handled. The latter facilitates the use of the data for generic purposes.

Normalised to the amount of active substance handled, the total potential dermal exposure was in the range of 0.407 mg/kg a.s. up to 5.27 mg/kg a.s.; inhalation was of minor importance *i.e.* <0.001 mg/kg a.s.).

With respect to prothioconazole, the actual dermal exposure during mixing/loading and application amounts to maximum 0.024 mg/kg a.s. (including correction for field recovery), inhalation exposure during mixing/loading and application amounts to 0.00078 mg/kg a.s.

Conversion of prothioconazole to prothioconazole -desthio was seen on garments, protective gloves and hands to various percentages up to about maximum 60% of the total applied prothioconazole.

The actual dermal exposure to prothioconazole -desthio during mixing/loading and application amounts to maximum 0.010 mg/kg a.s. (including correction for field recovery), inhalation exposure during mixing/loading and application amounts to 0.0007 mg/kg a.s.

Materials and methods

A. Materials

1. **Test Material:** Prothioconazole EC 250
   (alternative name: JAU 6476 250 EC)

   Lot/Batch No.: 06025/0259
   Purity: 250 g/L (prothioconazole)
   CAS No.: 178928-70-6 (prothioconazole)

2. **Study conditions:**

   Operation time: 28 - 50 mins (loading) [0.5 – 0.8 h]
   87 – 150 mins (application) [1.5 – 2.5 h]
   115 - 200 mins (total) [1.9 – 3.3 h]

Overall monitoring period: >5 - >7 h

Area treated 20 ha

Amount of a.s. applied: 3.96 - 4.06 kg

No. of tasks: 2 - 6 (loading)
2 - 6 (application)

3. Equipment used: Tractor (with cabin) trailed boom sprayer application.
28 m boom, 2500 L water tank volume

Tractor (with cabin) mounted boom sprayer application.
15 m boom, 800 L water tank volume

4. Environmental conditions:

Temperature: Measured but not reported

Humidity: Measured but not reported

Wind speed: Measured but not reported

B. Study Design:

1. In life dates: 5 May 2000 to 21 September 2000 (experimental dates)

2. Animal assignment and treatment: Mixer/loader/application study was undertaken to exposure to prothioconazole in the formulation, Prothioconazole EC 250 to cereal crops. In addition, exposure to prothioconazole the proportion of conversion to prothioconazole-desthio and the resulting exposure to prothioconazole-desthio was determined.

A total of 8 applications at three different spray timings involving three different operators were monitored. All participants were familiar with the practice of mixing/loading and application of plant protection products. All applications were performed during the actual season (May/June 2000) in Monheim, Germany.

With each application about 20 ha were treated using spray equipment that was appropriate and representative (tractor drawn/mounted ground boom sprayer). During the first two spray timings an equipment for larger field sizes was used (28 m boom, 2500 L water tank volume) whereas during the third spray timing an equipment for smaller field sizes was chosen (15 m boom, 800 L water tank volume). The tractors used were all equipped with a cabin.

The spraying lasted between 2.5 h and 3.5 h.

C. Methods:

1. Field recovery: Field recovery samples to assess the stability of prothioconazole and prothioconazole-desthio were performed on all sampling media exposed appropriately on each spraying occasion.

2. Body and head exposure: Dermal exposure of the body was determined via whole body underwear (long sleeved T-shirt, long johns) as well as by analysing a cotton shirt and a pair of trousers (cotton/polyester) as outer garments. Exposure to the head was determined using a cap. The results of the outer garments and the cap together with the results of the underwear correspond to potential dermal exposure of the body whereas the results of the underwear plus the cap are regarded as actual dermal body exposure when wearing one layer of clothing only.

3. Hand exposure: Determined via glove rinsing and hand washing. The results of the glove rinsing together with the hand washing correspond to potential hand exposure whereas the results of the hand washing are regarded as actual hand exposure. According to usual agricultural practice protective gloves were always worn during mixing/loading whereas during application gloves only would be worn if the operator had to handle contaminated surfaces, e.g. correcting a machine malfunction.

4. Inhalation exposure:	Determined by IOM-samplers equipped with glass fibre filters which were fixed to the garments at the breathing zone of the operator and connected to an individually powered air pump.
5. Termination of application:	At the end of the spraying procedure the cap and the gloves were sampled and also a hand wash was performed. The operators continued to wear the other dosimeter clothes further on to give a total of about 7 h (one exception: *ca*. 5.2 h) to provide some information on the proportion of conversion of prothioconazole to prothioconazole-desthio during the time of almost a full work day
6. Extraction and analysis:	Samples were extracted, followed by LC-MS/MS determination. The results of the measurements are reported as determined (*i.e.*, µg/sample) and as specific (normalised) exposure values, *i.e.*, as mg of exposure/kg of a.s/ handled. The latter facilitates the use of the data for generic purposes.

Results

A. Limit of quantification:

The limit of quantitation (LOQ) was 50 µg prothioconazole/sample for outer garments, 10 µg prothioconazole/sample for inner garments, 200 µg prothioconazole for one nitrile glove

(400 µg/pair), 0.01 µg prothioconazole /mL hand wash water (corresponding to 5 µg prothioconazole on hands) and 0.1 µg prothioconazole for glass fibre filters.

The corresponding LOQ for prothioconazole-desthio was 20 µg/sample for outer garments, 2 µg/sample for inner garments, 0.004 µg/mL hand wash water (corresponding to 2 µg on hands) and 0.1 µg for glass fibre filters.

For samples which showed results <LOQ the exposure values for prothioconazole and prothioconazole-desthio were then calculated from figures corresponding to half of the LOQ.

B. Measured amounts of prothioconazole:

Out of a total of 32 samples, eight samples of the outer clothing were found to have measurable amounts of prothioconazole; prothioconazole-desthio was quantified in four of these samples and in one additional sample. The % of conversion with respect to total 'prothioconazole-equivalents' was very variable, ranging from 3% to nearly 50%. Both prothioconazole and prothioconazole-desthio were found on gloves and in some of the hand wash solutions. The corresponding percentages of prothioconazole-desthio to total "prothioconazole equivalents" cover the range from 1 % to 60 %.

Spray tank samples which were also analysed showed that prothioconazole-desthio amounted from 0.1 % up to about 1 % of total 'prothioconazole-equivalents' maximum, with a mean of 0.22 %.

Prothioconazole-equivalents can be calculated by summing up the exposure figures for prothioconazole and prothioconazole-desthio, calculated as prothioconazole by taking into account the molar ratio (344.3 / 312.2 = 1.103). The resulting figures expressed as normalised dermal exposure values in mg/kg prothioconazole handled are listed in Table CP 7.2.1.2.1/01-1 to Table CP 7.2.1.2.1/01-3.

Table CP 7.2.1.2/01-1: Prothioconazole EC 250 mixer/loading/application exposure study: normalized dermal exposure to prothioconazole (in mg/kg prothioconazole)

Operator	Outer clothing	Under-garments	Head (cap)	Glove rinsings			Hand washing
				M/L	A	Total	
A1	0.069	0.0038	0.0063	0.422	-	0.422	0.0006
B1	0.025	0.0037	0.0062	1.23	-	1.23	0.0006
C1	0.032	0.0037	0.0062	0.878	-	0.878	0.0006
B2	0.025	0.0037	0.0062	0.407	-	0.407	0.0006
C2	0.115	0.0037	0.0062	3.55	-	3.55	0.0006
A3	0.102	0.0037	0.0062	2.66	-	2.66	0.0018
C3	0.039	0.0037	0.0062	5.27	-	5.27	0.0020
B3	0.025	0.0037	0.0062	3.42	-	3.42	0.0012

Table CP 7.2.1.2/01-2: Prothiconazole EC 250 mixer/loading/application exposure study: normalized dermal exposure to prothioconazole-desthio (in mg/kg prothioconazole)

Operator	Outer clothing	Under-garments	Head (cap)	Glove rinsings			Hand washing
				M/L	A	Total	
A1	0.019	0.0008	0.0025	0.003	-	0.003	0.0003
B1	0.010	0.0007	0.0025	0.008	-	0.008	0.0002
C1	0.010	0.0007	0.0025	0.007	-	0.007	0.0002
B2	0.010	0.0007	0.0025	0.021	-	0.021	0.0002
C2	0.010	0.0007	0.0025	0.050	-	0.050	0.0002
A3	0.018	0.0007	0.0025	0.112	-	0.112	0.0005
C3	0.013	0.0007	0.0025	0.185	-	0.185	0.0005
B3	0.012	0.0007	0.0025	0.151	-	0.151	0.0018

Only prothioconazole was found in two filters following inhalation exposure assessment. In both samples, the amount of prothioconazole was at the level of the LOQ (0.1 µg/sample) for this matrix, with corresponding normalized exposure values expressed as µg/kg prothioconazole handled are presented in Table CP 7.2.1.2/01-3

Table CP 7.2.1.2/01-3: Prothiconazole EC 250 mixer/loading/application exposure study: normalized inhalation exposure to prothioconazole and prothioconazole-desthio (in µg/kg prothioconazole)

	prothioconazole		Prothioconazole-desthio	
Operator	M/L	A	M/L	A
A1	0.35	0.35	0.35	0.35
B1	0.43	0.35	0.35	0.35
C1	0.35	0.35	0.35	0.35
B2	0.43	0.35	0.35	0.35
C2	0.35	0.35	0.35	0.35
A3	0.17	0.17	0.17	0.17
C3	0.17	0.17	0.17	0.17
B3	0.17	0.17	0.17	0.17

C. Deficiencies:

None.

Assessment and conclusion by applicant:

Assessment: This study is deemed acceptable and meets the requirements in 284/2013.

Conclusion: With respect to prothioconazole, the actual dermal exposure during mixing/loading and application amounts to maximum 0.024 mg/kg a.s. (including correction for field recovery), inhalation exposure during mixing/loading and application amounts to 0.00078 mg/kg a.s.

Conversion of prothioconazole to prothioconazole-desthio was seen on garments, protective gloves and hands to various percentages up to about maximum 60%.

The actual dermal exposure to prothioconazole-desthio during mixing/loading and application amounts to maximum 0.010 mg/kg a.s. (including correction for field recovery), inhalation exposure during mixing/loading and application amounts to 0.0007 mg/kg a.s.

Data Point:	KCP 7.2.1.2/02
Report Author:	[redacted]; [redacted]
Report Year:	2007
Report Title:	Determination of exposure during mixing/loading and application of Proline in cereals
Report No:	MR-156/05
Document No:	M-285798-01-1
Guideline(s) followed in study:	OECD guidance document for the conduct of studies of occupational exposure to pesticides during agricultural application, Series on Testing and Assessment No 9, 1997
Deviations from current test guideline:	None
Previous evaluation:	yes, evaluated and accepted RAR (2010)
GLP/Officially recognised testing facilities:	Yes, conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	Yes

Executive summary

An operator exposure study was carried out to measure the exposure of operators to prothioconazole and to its degradation product prothioconazole-desthio when applying Proline® to cereals. The applications were performed during the actual season on fields surrounding Limburg, Büdingen and Darmstadt (Germany). The areas treated ranged from 19 ha to 67 ha, with five applications involving five different operators. Tractor mounted boom spray applications were used, this was split between a 15 m boom and 1000 L water tank volume used by three operators (typically used for small field applications) and two operators using a 18/30 m boom, with 3000/4000 L water tank volume (typically used for large field applications). In all cases, tractors were equipped with cabins. However, depending on the weather and the equipment some operators left the back and/or front window open as well as the roof opening.

Dermal exposure of the body was determined *via* whole body underwear (long sleeved T-shirt, long johns) as well as by analysing a cotton shirt and a pair of trousers (cotton/polyester) as outer garments. Exposure to the head was determined using a cap. The results of the outer garments and the cap together with the results of the underwear are regarded as potential dermal exposure of the body whereas the results of the underwear plus the cap are regarded as actual dermal exposure when wearing one layer of clothing only. The operators were not forced to wear a cap if this was not in accordance to their normal working clothes and behaviour. One operator made use of this option.

Hand exposure was determined *via* glove rinsing and hand washing. The results of the glove rinsing together with the hand washing are regarded as potential hand exposure whereas the results of the hand washing are regarded as actual hand exposure. According to usual agricultural practice protective gloves were always worn during mixing loading whereas during application gloves were only worn if the operator had to handle contaminated surfaces, *e.g.* un-/folding the boom manually or correcting a machine malfunction like blocked or lost nozzles.

Inhalation exposure was determined by use of a personal air sampling pump connected to an IOM-sampler with glass fibre filter, located in the breathing zone of the operator.

The spraying lasted between 6 hours and 9 hours. On completion of spraying the cap and the gloves were sampled and also a hand wash was performed. At the end of the last application, the operators removed the other dosimeter clothes to provide information on the proportion of conversion of prothioconazole to prothioconazole-desthio during the time of almost a full work day.

Samples were extracted, followed by LC-MS/MS determination. The results of the measurements are reported as determined (*i.e.*, µg a.s./sample) and as specific (normalized) exposures, *i.e.*, as mg of exposure/kg of a.s. handled. The latter facilitates the use of the data for generic purposes.

Normalised to the amount of active substance handled, the total potential dermal exposure was in the range of 0.047 mg/kg a.s. up to 0.999 mg/kg a.s.; inhalation was of minor importance.

With respect to prothioconazole, the actual dermal exposure during mixing/loading and application With respect to prothioconazole, the actual dermal exposure (under garments, head, hand washing) during mixing/loading and application amounts to maximum 1.0118 mg/kg a.s. (including correction for field recovery), inhalation exposure during mixing/loading and application amounts to 0.00018 mg/kg a.s.

Conversion of prothioconazole to prothioconazole -desthio was seen on garments, protective gloves and hands to various percentages up to about maximum 60%.

The actual dermal exposure to prothioconazole-desthio during mixing/loading and application amounts to maximum 0.0041 mg/kg a.s. (including correction for field recovery), inhalation exposure during mixing/loading and application amounts to 0.00016 mg/kg a.s.

Materials and methods

A. Materials

1. Test Material:	Prothioconazole EC 250 (alternative name: Proline® 250 EC)
Lot/Batch No.:	PF90102896, PF90101837, PF900092233, PF90102189
Purity:	248-252.5 g/L (prothioconazole)
CAS No.:	178928-70-6 (prothioconazole)
2. Study conditions:	
Operation time:	13 – 87 mins (loading) [0.2 – 1.5 h] 242 – 312 mins (application) [4.0 - 5.2 h] 294 – 483 mins (total) [4.9 – 8.1 h]
Monitoring time	6 – 9 h
Area treated	18.9 – 67.4 ha
Amount of a.s. applied:	4.0 – 13.6 kg
No. of tasks:	3 – 7 (loading) 3 – 6 (application)
3. Equipment used:	Tractor (with cabin) drawn boom sprayer application. 15 m boom, 1000 L water tank volume 30 m boom, 4000 L water tank volume Tractor (with cabin) mounted boom sprayer application. 15 m boom, 1000 L water tank volume 15 m boom, 1100 L water tank volume
4. Environmental conditions:	
Temperature:	8 – 30°C (across 5 test sites)
Humidity:	34 – 64% (across 5 test sites)
Wind speed:	1 – 4 m/s

B. Study Design:

1. In life dates:	12 May 2000 to 16 June 2005 (experimental dates)
2. Animal assignment and treatment:	Mixer/loader/application study was undertaken to exposure to prothioconazole in the formulation, Prothioconazole EC 250 to cereal crops. In addition, exposure to prothioconazole the proportion of conversion to prothioconazole-desthio and the resulting exposure to prothioconazole-desthio was determined. A total of 5 applications at five different spray timings involving five different ♂ operators were monitored. All participants were familiar with the practice of

mixing/loading and application of plant protection products. All applications were performed during the actual season (May/June 2000) in, Germany.

With each application about 19-67 ha were treated using spray equipment that was appropriate and representative (tractor drawn/mounted ground boom sprayer). Tractor mounted boom spray applications were used, this was split with a 15 m boom and 1000 L water tank volume used by three operators (typically used for small field applications) and two operators using a 18/30 m boom, with 3000/4000 L water tank volume (typically used for large field applications). In all cases, tractors were equipped with cabins. However, depending on the weather and the equipment some operators left the back and/or front window open as well as the roof opening

The overall monitoring period lasted between 6 h and 9 h.

C. Methods:

1. **Field recovery:** Field recovery samples to assess the stability of prothioconazole and prothioconazole-desthio were performed on all sampling media exposed appropriately on each spraying occasion.

2. **Body and head exposure:** Dermal exposure of the body was determined via whole body underwear (long sleeved T-shirt, long johns) as well as by analysing a cotton shirt and a pair of trousers (cotton/polyester) as outer garments. Exposure to the head was determined using a cap. The results of the outer garments and the cap together with the results of the underwear correspond to potential dermal exposure of the body whereas the results of the underwear plus the cap are regarded as actual dermal body exposure when wearing one layer of clothing only.

3. **Hand exposure:** Determined via glove rinsing and hand washing. The results of the glove rinsing together with the hand washing correspond to potential hand exposure whereas the results of the hand washing are regarded as actual hand exposure. According to usual agricultural practice protective gloves were always worn during mixing/loading whereas during application gloves only would be worn if the operator had to handle contaminated surfaces.

4. **Inhalation exposure:** Determined by IOM-samplers equipped with glass fibre filters which were fixed to the garments at the breathing zone of the operator and connected to an individually powered air pump.

5. **Termination of application:** At the end of the spraying procedure the cap and the gloves were sampled and also a hand wash was performed. On completion of spraying the cap and the gloves were sampled and also a hand wash was performed. At the end of the last application, the operators removed the other dosimeter clothes to provide information on the proportion of conversion of prothioconazole to prothioconazole-desthio during the time of almost a full work day.

6. **Extraction and analysis:** Samples were extracted, followed by LC-MS/MS determination. The results of the measurements are reported as determined (*i.e.*, µg/sample) and as specific (normalised) exposure values, *i.e.*, as mg of exposure/kg of a.s/ handled. The latter facilitates the use of the data for generic purposes.

Results

A. Limit of quantification:

The limit of quantitation (LOQ) was 50 µg prothioconazole/sample for outer garments, 10 µg prothioconazole/sample for inner garments, 200 µg prothioconazole for one nitrile glove

(400 µg/pair), 0.01 µg prothioconazole /mL hand wash water (corresponding to 5 µg prothioconazole on hands) and 0.1 µg prothioconazole for glass fibre filters.

The corresponding LOQ for prothioconazole-desthio was 20 µg/sample for outer garments, 2 µg/sample for inner garments, 0.004 µg/mL hand wash water (corresponding to 2 µg on hands) and 0.1 µg for glass fibre filters.

For samples which showed results <LOQ the exposure values for prothioconazole and prothioconazole-desthio were then calculated from figures corresponding to half of the LOQ.

B. Measured amounts of prothioconazole:

On 12 samples of the outer clothing measurable amounts of prothioconazole were found; in eight of these samples and in one additional sample also prothioconazole-desthio could be quantified (out of a total of 24 samples). The % of conversion with respect to total 'prothioconazole-equivalents' was very variable, ranging from 5% to nearly 56%. Also on gloves and in some of the hand wash solutions prothioconazole and prothioconazole-desthio were found. The corresponding percentages of prothioconazole-desthio to total "prothioconazole equivalents" cover the range from 3% to 60 %.

Prothioconazole-equivalents can be calculated by summing up the exposure figures for prothioconazole and prothioconazole-desthio, calculated as prothioconazole by taking into account the molar ratio (344.3 / 312.2 = 1.103). The resulting figures expressed as normalised dermal exposure values in mg/kg prothioconazole handled are listed in Table CP 7.2.1.2.1/02-1 to Table CP 7.2.1.2.1/02-3.

Table CP 7.2.1.2/02-1: Prothioconazole EC 250 mixer/loading/application exposure study: normalized dermal exposure to prothioconazole (in mg/kg prothioconazole)

Operator	Outer clothing	Under-garments	Head (cap)	Glove rinsings			Hand washing
				M/L	A	Total	
A	0.111	0.0011	0.00018	0.686	<0.001	0.686	0.00093
B	0.040	0.0038	0.0063	0.690	0.229	0.919	0.0019
C	0.285	0.0043	0.0071	0.999	-	0.999	0.0014
D	0.010	0.0011	0.0019	0.047	0.046	0.093	0.0008
E	0.031	0.0028	0.0047	0.285	-	0.285	0.0014

Table CP 7.2.1.2.1/02-2: Prothioconazole EC 250 mixer/loading/application exposure study: normalized dermal exposure to prothioconazole-desthio (in mg/kg prothioconazole)

Operator	Outer clothing	Under-garments	Head (cap)	Glove rinsings			Hand washing
				M/L	A	Total	
A	0.034	0.0003	0.0007	0.045	<0.001	0.045	0.0088
B	0.018	0.0008	0.0025	0.062	0.025	0.087	0.0011
C	0.029	0.0009	0.0029	0.073	-	0.073	0.0003
D	0.005	0.0002	0.0008	0.004	0.009	0.013	0.0003
E	0.008	0.0006	0.0019	0.016	-	0.016	0.0006

Only prothioconazole was found in two filters following inhalation exposure assessment. In both samples, the amount of prothioconazole was at the level of the LOQ (0.1 µg/sample) for this matrix, with corresponding normalized exposure values expressed as µg/kg prothioconazole handled are presented in Table CP 7.2.1.2/02-3

Table CP 7.2.1.2/02-3: Prothioconazole EC 250 mixer/loading/application exposure study: normalized inhalation exposure to prothioconazole and prothioconazole-desthio (in µg/kg prothioconazole)

	prothioconazole		Prothioconazole-desthio	
Operator	M/L	A	M/L	A
A	0.10	0.05	0.03	0.03
B	0.09	0.09	0.09	0.09
C	0.10	0.06	0.10	0.06
D	0.03	0.03	0.03	0.03
E	0.07	0.07	0.07	0.07

C. Deficiencies:

None.

Assessment and conclusion by applicant:

Assessment: This study is deemed acceptable and meets the requirements in 284/2013.

Conclusion: With respect to prothioconazole, the actual dermal exposure (under garments, head, hand washing) during mixing/loading and application amounts to maximum 1.0118 mg/kg a.s. (including correction for field recovery), inhalation exposure during mixing/loading and application amounts to 0.00018 mg/kg a.s.

Conversion of prothioconazole to prothioconazole-desthio was seen on garments, protective gloves and hands to various percentages up to about maximum 60%.

The actual dermal exposure to prothioconazole-desthio during mixing/loading and application amounts to maximum 0.0041 mg/kg a.s. (including correction for field recovery), inhalation exposure during mixing/loading and application amounts to 0.00016 mg/kg a.s.

Data Point:	KCP 7.2.1.2/03
Report Author:	[redacted]; [redacted]
Report Year:	2007
Report Title:	Determination of exposure during mixing/loading and application of prothioconazole in cereals and canola
Report No:	MR-244/07
Document No:	M-286545-01-1
Guideline(s) followed in study:	OECD guidance document for the conduct of studies of occupational exposure to pesticides during agricultural application. Series on Testing and Assessment No. 9, 1997
Deviations from current test guideline:	None
Previous evaluation:	yes, evaluated and accepted RAR (2010)
GLP/Officially recognised testing facilities:	Yes, conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	Yes

Executive summary

An operator exposure study was carried out to measure the exposure of operators to prothioconazole and to its degradation product prothioconazole-desthio when applying Input® to cereals and Proline® to canola. The applications were performed during the actual season on fields surrounding Weimar/Gera and Swisttal/Weilerswist (Germany). The areas treated ranged from 23 ha to 180 ha, with seven applications involving seven different operators. Tractor mounted boom spray applications were used, this was split between a 15/21 m boom and 840 - 1500 L water tank volume used by three operators (typically used for small field applications) and four operators using a 24/36 m boom, with 2600/4000 L water tank volume (typically used for large field applications). In all cases, tractors were equipped with cabins. However, depending on the weather and the equipment some operators left the back and/or front window open as well as the roof opening.

Dermal exposure of the body was determined *via* whole body underwear (long sleeved T-shirt, long johns) as well as by analysing a cotton shirt and a pair of trousers (cotton/polyester) as outer garments. Exposure to the head was determined using a cap. The results of the outer garments and the cap together with the results of the underwear are regarded as potential dermal exposure of the body whereas the results of the underwear plus the cap are regarded as actual dermal exposure when wearing one layer of clothing only. The operators were not forced to wear a cap if this was not in accordance to their normal working clothes and behaviour. One operator made use of this option.

Hand exposure was determined *via* glove rinsing and hand washing. The results of the glove rinsing together with the hand washing are regarded as potential hand exposure whereas the results of the hand washing are regarded as actual hand exposure. According to usual agricultural practice protective gloves

were always worn during/mixing loading whereas during application gloves were only worn in the case the operator had to handle contaminated surfaces, *e.g.* un-/folding the boom manually or correcting a machine malfunction like blocked or lost nozzles.

Inhalation exposure was determined by use of a personal air sampling pump connected to an IOM-sampler with glass fibre filter, located in the breathing zone of the operator.

The spraying lasted between 5 hours and 9 hours. On completion of spraying the cap and the gloves were sampled and also a hand wash was performed. At the end of the last application, the operators removed the other dosimeter clothes to provide information on the proportion of conversion of prothioconazole to prothioconazole-desthio during the time of almost a full work day.

Samples were extracted, followed by LC-MS/MS determination. The results of the measurements are reported as determined (*i.e.*, µg a.s./sample) and as specific (normalized) exposures, *i.e.*, as mg of exposure/kg of a.s. handled. The latter facilitates the use of the data for generic purposes.

Normalised to the amount of active substance handled, the total potential dermal exposure was in the range of 0.040 mg/kg a.s. up to 0.836 mg/kg a.s.; inhalation was of minor importance.

With respect to prothioconazole, the actual dermal exposure (under garments, head, hand washing) during mixing/loading and application amounts to maximum 0.0148 mg/kg a.s. (including correction for field recovery), inhalation exposure during mixing/loading and application amounts to 0.00043 mg/kg a.s.

Conversion of prothioconazole to prothioconazole -desthio was seen on garments, protective gloves and hands to various percentages up to about maximum 72%.

The actual dermal exposure to prothioconazole-desthio during mixing/loading and application amounts to maximum 0.0041 mg/kg a.s. (including correction for field recovery), inhalation exposure during mixing/loading and application amounts to 0.00030 mg/kg a.s.

Materials and methods

A. Materials

1. Test Material:	Prothioconazole EC 250 (alternative name: Proline® 250 EC)	Prothioconazole EC 460 (alternative name: Proline® 250 EC)
Lot/Batch No.:	EM4L002817	PF90131479
Purity:	248 g/L (prothioconazole)	160.8 g/L (prothioconazole)
CAS No.:	178928-70-6 (prothioconazole)	
2. Study conditions:		
Operation time:	56 – 143 mins (loading) [0.9 – 2.4 h] 231 – 377 mins (application) [3.9 – 6.3 h]	
Monitoring time	287 - 515 mins (total) [4.8 – 8.4 h]	
Area treated	23 - 80 ha (with one area of 180 ha)	
Amount of a.s. applied:	4.6 - 31.3 kg a.s	
Application volume:	200 – 300 L/ha	
No. of tasks:	4 – 14 (loading) 4 – 14 (application)	
3. Equipment used:	Tractor (with cabin) mounted boom sprayer application: 15 m boom, 840 L water tank volume 15 m boom, 1000 L water tank volume 21 m boom, 1500 L water tank volume Tractor (with cabin) self propelled boom sprayer application: 24 m boom, 2600 L water tank volume	

24 m boom, 4000 L water tank volume
36 m boom, 4000 L water tank volume

Tractor (with cabin) drawn boom sprayer application:
24 m boom, 4000 L water tank volume

4. Environmental conditions:

Temperature: Measured but not reported

Humidity: Measured but not reported

Wind speed: Measured but not reported

B. Study Design:

1. In life dates: 5 May 2006 to 16 June 2006 (experimental dates)

2. Animal assignment and treatment: Mixer/loader/application study was undertaken to exposure to prothioconazole in the formulation, Prothioconazole EC 250 to cereal crops. In addition, exposure to prothioconazole the proportion of conversion to prothioconazole-desthio and the resulting exposure to prothioconazole-desthio was determined.

A total of 7 applications at seven different spray timings involving seven different 7 operators were monitored. All participants were familiar with the practice of mixing/loading and application of plant protection products. All applications were performed during the actual season (May/June 2006) in, Germany.

With each application about 23-180 ha were treated using spray equipment that was appropriate and representative (tractor drawn/mounted ground boom sprayer). Whilst tractor mounted boom spray applications were used, this was split with a 15/21 m boom and 840/1500 L water tank volume (typically used for small field applications) used by three operators and four operators using a 24/36 m boom, with 2600/4000 L water tank volume (typically used for large field applications). In all cases, tractors were equipped with cabins. However, depending on the weather and the equipment some operators left the back and/or front window open as well as the roof opening

The spraying lasted between 5 h and 9 h.

C. Methods:

1. Field recovery: Field recovery samples to assess the stability of prothioconazole and prothioconazole-desthio were performed on all sampling media exposed appropriately on each spraying occasion.

2. Body and head exposure: Dermal exposure of the body was determined via whole body underwear (long sleeved T-shirt, long johns) as well as by analysing a cotton shirt and a pair of trousers (cotton/polyester) as outer garments. Exposure to the head was determined using a cap. The results of the outer garments and the cap together with the results of the underwear correspond to potential dermal exposure of the body whereas the results of the underwear plus the cap are regarded as actual dermal body exposure when wearing one layer of clothing only.

3. Hand exposure: Determined via glove rinsing and hand washing. The results of the glove rinsing together with the hand washing correspond to potential hand exposure whereas the results of the hand washing are regarded as actual hand exposure. According to usual agricultural practice protective gloves were always worn during mixing/loading whereas during application gloves only would be worn if the operator had to handle contaminated surfaces.

4. Inhalation exposure: Determined by IOM-samplers equipped with glass fibre filters which were fixed to the garments at the breathing zone of the operator and connected to an individually powered air pump.

5. Termination of application: At the end of the spraying procedure the cap and the gloves were sampled and also a hand wash was performed. On completion of spraying the cap and the gloves were sampled and also a hand wash was performed. At the end of the last application, the operators removed the other dosimeter clothes to provide

	information on the proportion of conversion of prothioconazole to prothioconazole-desthio during the time of almost a full work day.
6. Extraction and analysis:	Samples were extracted, followed by LC-MS/MS determination. The results of the measurements are reported as determined (*i.e.*, µg/sample) and as specific (normalised) exposure values, *i.e.*, as mg of exposure/kg of a.s/ handled. The latter facilitates the use of the data for generic purposes.

Results

A. Limit of quantification:

The limit of quantitation (LOQ) per sample was 50 µg (outer garments), 10 µg (undergarments) and 5 µg (hand wash water) for prothioconazole and 20 µg, 2 µg and 2 µg for prothioconazole-desthio, respectively. Exposure values for samples which showed results < LOQ for prothioconazole and prothioconazole-desthio were calculated using figures corresponding to half of the LOQ.

B. Measured amounts of prothioconazole:

On 18 samples of the outer clothing measurable amounts of prothioconazole were found and in 17 samples also prothioconazole-desthio could be quantified (out of a total of 28 samples). The percentage of conversion with respect to total 'prothioconazole-equivalents' was very variable, ranging from 2% to 77%. In three samples of the undergarments prothioconazole was found and in four samples prothioconazole-desthio. The corresponding percentage of conversion was in the range of 5% to 52%. Also on gloves and in some of the hand wash solutions prothioconazole and prothioconazole-desthio were found. The corresponding percentages of prothioconazole-desthio to total 'prothioconazole equivalents' cover the range from 2% to 72%.

Prothioconazole-equivalents can be calculated by summing up the exposure figures for prothioconazole and prothioconazole-desthio, calculated as prothioconazole by taking into account the molar ratio (344.3 / 312.2 = 1.103). The resulting figures expressed as normalised dermal exposure values in mg/kg prothioconazole handled are listed in Table CP 7.2.1.2/03-1 to Table CP 7.2.1.2/03-3.

Table CP 7.2.1.2/03-1: Prothioconazole EC 250 mixer/loading/application exposure study: normalized dermal exposure to prothioconazole (in mg/kg prothioconazole)

Operator	Outer clothing	Under-garments	Head (cap)	Glove rinsings			Hand washing
				M/L	A	Total	
A	0.035	0.0063	0.0054	0.687	0.041	0.728	0.0031
B	0.025	0.0012	0.0020	0.606	-	0.606	0.0012
C	0.156	0.0007	0.0008	0.095	-	0.095	0.0004
D	0.012	0.0013	0.0021	0.040	-	0.040	0.0004
E	0.148	0.0027	0.0045	0.243	0.293	0.536	0.0017
F	0.042	0.0021	0.0035	0.834	0.002	0.836	0.0011
H	0.225	0.0017	0.0017	0.220	-	0.220	0.0003

Table CP 7.2.1.2/03-2: Prothioconazole EC 250 mixer/loading/application exposure study: normalized dermal exposure to prothioconazole-desthio (in mg/kg prothioconazole)

Operator	Outer clothing	Under-garments	Head (cap)	Glove rinsings			Hand washing
				M/L	A	Total	
A	0.036	0.0001	0.0022	0.072	0.020	0.092	0.0007
B	0.006	0.0002	0.0008	0.014	-	0.014	0.0011
C	0.017	0.0003	0.0003	0.003	-	0.003	0.0006
D	0.005	0.0003	0.0008	0.006	-	0.006	0.0002
E	0.053	0.0010	0.0018	0.05	0.053	0.068	0.0013
F	0.010	0.0007	0.0014	0.041	0.004	0.045	0.0004
H	0.007	0.0002	0.0007	0.013	-	0.013	0.0004

Only prothioconazole was found in two filters following inhalation exposure assessment. In both samples, the amount of prothioconazole was at the level of the LOQ (0.1 µg/sample) for this matrix,

with corresponding normalized exposure values expressed as µg/kg prothioconazole handled are presented in Table CP 7.2.1.2/03-3

Table CP 7.2.1.2/03-3: Prothiconazole EC 250 mixer/loading/application exposure study: normalized inhalation exposure to prothioconazole and prothioconazole-desthio (in µg/kg prothioconazole)

	prothioconazole		Prothioconazole-desthio	
Operator	**M/L**	**A**	**M/L**	**A**
A	0.15	0.15	0.15	0.15
B	0.05	0.25	0.05	0.05
C	0.02	0.02	0.02	0.02
D	0.06	0.06	0.06	0.06
E	0.13	0.30	0.13	0.13
F	0.10	0.20	0.10	0.10
H	0.05	0.05	0.05	0.05

C. Deficiencies:

None.

Assessment and conclusion by applicant:

Assessment: This study is deemed acceptable and meets the requirements in 284/2013.

Conclusion: With respect to prothioconazole, the actual dermal exposure (under garments, head, hand washing) during mixing/loading and application amounts to maximum 0.0148 mg/kg a.s. (including correction for field recovery), inhalation exposure during mixing/loading and application amounts to 0.00043 mg/kg a.s.

Conversion of prothioconazole to prothioconazole-desthio was seen on garments, protective gloves and hands to various percentages up to about maximum 72%.

The actual dermal exposure to prothioconazole-desthio during mixing/loading and application amounts to maximum 0.0041 mg/kg a.s. (including correction for field recovery), inhalation exposure during mixing/loading and application amounts to 0.00030 mg/kg a.s.

Overall conclusion of operator exposure studies examing prothioconazole and prothioconazole-desthio

In total, three operator exposure studies have been undertaken with these conducted as mixer/loader/application studies. These studies were undertaken by professional operators in Europe, reflective of real work situations. Tractor mounted or boom spray application to cereal crops was used, with the work conducted between 2000 – 2006. These studies evaluated the exposure of operators to prothioconazole and to its degradation product prothioconazole-desthio when applying Prothioaconazole EC 250 formulation (3 occasions) or EC 460 formulation (1 occasion, conducted in conjunction with EC 250 application).

Treated areas ranged from 20 ha (conducted in 2000, prior to approval for prothioconazole, therefore treated areas restricted) through to typically *ca* 80 ha (on a single occasion an area of 180 ha was treated, with all these areas treated following national approval). In total, 20 replicates, performed by 15 operators were monitored. In all cases, tractor cabins were installed (as it standard practice).

Table CP 7.2.1.2-4: Collective overview of study parameters from all three operator studies

Assigned operator ID	Area treated (ha)	Equipment utilized	No. of tasks (load/application)	Reference

A1	20	Tractor-drawn boom, 28 m, 2500 L spray tank	2/2	CP 7.2.1.2/01 M-040604-01-1
B1	20		2/2	
C1	20		2/2	
B2	20		2/2	
C2	20		2/2	
A3	20	Tractor-mounted boom, 15 m, 800 L spray tank	6/6	
C3	20		6/6	
B3	20		6/6	
A	67	Tractor-drawn boom, 30 m, 4000 L spray tank	4/4	CP 7.2.1.2/02 [redacted] (2002) M-285798-01-1
B	19	Tractor-mounted boom, 15 m (manual folding), 1000 L spray tank	4/4	
C	33	Tractor-mounted boom, 15 m, 1100 L spray tank	7/6	
D	49	Tractor-drawn boom, 15 m, 3000 L spray tank	3/3	
E	25	Tractor-mounted boom, 15 m, 1000 L spray tank	6/6	
A	23	Tractor-mounted boom, 15 m (manual folding), 840 L spray tank	9/9	CP 7.2.1.2/03 M-286545-01-1
B	64	Self-propelled 24 m boom, 4000 L spray tank	4/4	
C	180	Self-propelled 36 m boom, 4000 L spray tank	14/14	
D	60	Self-propelled 24 m boom, 4000 L spray tank	6/6	
E	30	Tractor-mounted boom, 15 m (manual folding), 1000 L spray tank	6/6	
F	35	Tractor-mounted boom, 21 m, 1500 L spray tank	8/8	
H	80	Tractor-mounted boom, 24 m, 4000 L spray tank	4/4	

The normalized exposure figures from all studies are listed in Table CP 7.2.1.2/01-2 for the exposure to prothioconazole and in Table CP 7.2.1.2/01-3 for the exposure to prothioconazole-desthio. Normalisation was performed with regard to the amount of active substance (= prothioconazole) handled.

To distinguish the results where all sample analyses resulted in a figure "<LOQ" from those where at least one of the samples showed a measurable result, the normalized figures which include at least one measurable residue are printed in bold and are shadowed in grey.

The LOQ per sample was 50 µg (outer garments), 10 µg (undergarments) and 5 µg (hand wash water) for prothioconazole and 20 µg, 2 µg and 2 µg for prothioconazole-desthio, respectively.

Table CP 7.2.1.2-5: Collective overview of study parameters from all three operator studies: prothioconazole dermal exposure

Assigned operator ID	Normalised dermal exposure (mg/kg a.s.)					Reference
	Outer clothing	Under-garments	Cap (head)	Gloves (M/L+A)	Hand rinsing	
A1	**0.069**	0.0038	0.0063	**0.422**	0.0006	CP 7.2.1.2/01 M-040604-01-1
B1	0.025	0.0037	0.0062	**1.23**	0.0006	
C1	**0.032**	0.0037	0.0062	**0.878**	0.0006	
B2	0.025	0.0037	0.0062	**0.407**	0.0006	
C2	**0.115**	0.0037	0.0062	**3.55**	**0.0018**	
A3	**0.102**	0.007	0.0062	**2.66**	0.0012	
C3	**0.039**	0.0037	0.0062	**5.27**	**0.0020**	

B3	0.025	0.0037	0.0062	**3.42**	0.0012	
A	**0.111**	0.0011	0.0018	**0.686**	**0.0093**	CP 7.2.1.2/02 M-285798-01-1
B	**0.040**	0.0038	0.0063	**0.919**	0.0019	
C	**0.2852**	0.0043	0.0071	**0.999**	0.0014	
D	**0.010**	0.0011	0.0019	**0.093**	0.0008	
E	**0.031**	0.0028	0.0047	**0.285**	0.0014	
A	**0.035**	**0.0063**	0.0054	**0.728**	**0.0031**	CP 7.2.1.2/03 M-286545-01-1
B	**0.025**	0.0012	0.0020	**0.606**	0.0012	
C	**0.156**	**0.0007**	0.0008	**0.095**	**0.0004**	
D	**0.012**	0.0013	0.0021	**0.040**	0.0004	
E	**0.148**	0.007	0.0045	**0.536**	**0.0017**	
F	**0.042**	0.0021	0.0035	**0.836**	0.0011	
H	**0.225**	**0.0017**	0.0017	**0.220**	0.0003	

The results show that on outer clothing the exposure to prothioconazole and prothioconazole-desthio covers a range of a factor of 28 (0.010 – 0.285 mg/kg as) and 11 (0.005 – 0.053 mg/kg a.s.), respectively. As the lower end figures are also derived from measured residues the spread reflects the differences in exposure.

For the undergarments representing the skin of the body the range of exposure to prothioconazole amounts to a factor of 9 (0.0007 – 0.0063 mg/kg a.s.), again, derived from measured residues. For prothioconazole-desthio this range amounts to a factor of 3 when including only measured residues or to a factor of 5 when including all results (0.0002/0.0003 – 0.0010 mg/kg as).

Table CP 7.2.1.2-6: Collective overview of study parameters from all three operator studies: prothioconazole-desthio dermal exposure

Assigned operator ID	Normalised dermal exposure (mg/kg a.s.)					Reference
	Outer clothing	Under-garments	Cap (head)	Gloves (M/L+A)	Hand rinsing	
A1	**0.019**	0.0008	0.0025	**0.003**	0.0003	CP 7.2.1.2/01 M-040604-01-1
B1	0.010	0.0007	0.0025	**0.008**	0.0002	
C1	0.010	0.0007	0.0025	**0.007**	0.0002	
B2	0.010	0.0007	0.0025	**0.021**	0.0002	
C2	0.010	0.0007	0.0025	**0.020**	0.0002	
A3	**0.018**	0.0007	0.0025	**0.112**	0.0002	
C3	**0.013**	0.0007	0.0025	**0.185**	0.0005	
B3	**0.012**	0.0007	0.0025	**0.151**	**0.0018**	
A	**0.034**	**0.0003**	0.0007	**0.045**	**0.0088**	CP 7.2.1.2/02 M-285798-01-1
B	**0.018**	0.0008	0.0025	**0.087**	0.0011	
C	**0.029**	0.0009	0.0029	**0.073**	0.0006	
D	**0.005**	0.0002	0.0008	**0.013**	0.0003	
E	0.008	0.0006	0.0019	**0.016**	0.0006	
A	**0.036**	0.0007	0.0022	**0.092**	0.0007	CP 7.2.1.2/03 M-286545-01-1
B	**0.006**	0.0002	0.0008	**0.014**	**0.0011**	
C	**0.017**	**0.0003**	0.0003	**0.003**	**0.0006**	
D	**0.005**	0.0003	0.0008	**0.006**	0.0002	
E	**0.053**	**0.0010**	0.0018	**0.068**	**0.0013**	
F	**0.010**	0.0004	0.0014	**0.045**	0.0004	
H	**0.007**	0.0002	0.0007	**0.013**	**0.0004**	

It is prudent to acknowledge that 17 out of 20 replicates had measurable residues of prothioconazole on their outer clothing with the remaining three operators showing measurable residues of prothioconazole on their undergarments. For prothioconazole-desthio in 15 out of 20 replicates, measurable residues were found on the outer clothing but only three operators showed measurable residues on their undergarments. Only one of the operators had measured residues of both prothioconazole and prothioconazole-desthio concurrently on his undergarments.

Exposure of the head (determined using a cap) was determined for 15 out of 20 replicates. In all cases for prothioconazole as well as for prothioconazole-desthio, the results were "< LOQ". Hence, it is acceptable that these results can also be extrapolated to the other five replicates to calculate a hypothetical head exposure. The highest figure for head exposure ("theoretical exposure" as derived from "< LOQ") results from an operator that has worn a cap during the study (CP 7.2.1.2/02 [M-285798-01-1], operator C).

The results of the protective gloves show higher exposure figures for the first study as compared to the second and the third. The reason for this is mainly due to the fact that most of the farmers in study 02 and study 03 (who had the possibility) rinsed the gloves under water before taking them off. This is in accordance with good occupational hygiene practice and therefore, any farmer who was going to behave like this was let to proceed as he was used to.

In addition, residues on protective gloves should be regarded to have an indicative character, similar to the residues on outer clothing or estimates of potential dermal exposure. Essential figures for risk assessments should relate to real actual dermal exposure data whenever they are available. With regard to actual hand exposure a range covering a factor of 23 for prothioconazole (0.0004 – 0.0093 mg/kg as) is found reflecting measured residues. For prothioconazole-desthio the range amounts to a factor of 22 resulting from measured residues or 44 when including all results (0.0002/0.0004 – 0.0088 mg/kg as).

Table CP 7.2.1.2-7: Collective overview of study parameters from all three operator studies: prothioconazole and prothioconazole-desthio inhalation exposure

Assigned operator ID	Normalised inhalation exposure (µg/kg a.s.)				Reference
	Prothioconazole		Prothioconazole-desthio		
	Mix/load	Application	Mix/load	Application	
A1	0.00035	0.00035	0.00035	0.00035	CP 7.2.1.2/01 M-040604-01-1
B1	**0.00043**	0.00035	0.00035	0.00035	
C1	0.00035	0.00035	0.00035	0.00035	
B2	**0.00043**	0.00035	0.00035	0.00035	
C2	0.00035	0.00035	0.00035	0.00035	
A3	0.00017	0.00017	0.00017	0.00017	
C3	0.00017	0.00017	0.00017	0.00017	
B3	0.00017	0.00017	0.00017	0.00017	
A	**0.00010**	**0.00005**	0.00003	0.00003	CP 7.2.1.2/02 M-285798-01-1
B	0.00009	0.00009	0.00009	0.00009	
C	0.00010	0.00006	0.00010	0.00006	
D	0.00003	0.00003	0.00003	0.00003	
E	0.00007	0.00007	0.00007	0.00007	
A	0.00015	0.00015	0.00015	0.00015	CP 7.2.1.2/03 M-286545-01-1
B	0.00005	**0.00025**	0.00005	0.00005	
C	0.00002	0.00002	0.00002	0.00002	
D	0.00006	0.00006	0.00006	0.00006	
E	0.00013	**0.00030**	0.00013	0.00013	
F	0.00010	**0.00020**	0.00010	0.00010	
H	0.00005	0.00005	0.00005	0.00005	

The inhalation results confirm that only prothioconazole was found and also only in a few replicates. The absolute residues of prothioconazole determined on the sampling devices were very low and did not exceed a level of four times the LOQ (0.1 µg/sample [0.0004 mg/sample). Prothioconazole-desthio was not found in any sample. Therefore, the variation in the normalised figures is only determined by the amount of active substance handled as well as by the number of sampling devices used (*e.g.* exchange of device per each work cycle in CP 7.2.1.2/01 [M-040604-01-1]) compared to one device only for mixing/loading and another for application in CP 7.2.1.2/02 [M-285798-01-1], CP 7.2.1.2/03 [M-286545-01-1]).

The percentage of conversion of prothioconazole to prothioconazole-desthio with respect to total "prothioconazole-equivalents" was found to be very variable on clothing, ranging from 2 % up to about 50 % with one single sample of 77 %. Also on gloves and in some of the hand wash solutions prothioconazole and prothioconazole-desthio were found. The corresponding percentages of prothioconazole-desthio to total "prothioconazole equivalents" cover the range from 1 % to about 70 % (a value of 50 % corresponds to the 90th percentile of the individual data approximately).

Whilst spray tank samples were only analysed in CP 7.2.1.2/01 [M-040604-01-1]), these data confirmed that prothioconazole is stable in the spray liquid. The conversion to the metabolite, prothioconazole-desthio was found in amounts from 0.1% to 1% of total "prothioconazole equivalents", with a mean of 0.22%.

Collectively, these data are in good agreement with the hypothesis that the formation of prothioconazole-desthio on surfaces is related to:

- the process of drying;
- the concentration of prothioconazole in a solution, and
- the nature of the surface on which prothioconazole is applied and dries

It however is important to put the percentage of conversion into perspective with regard to the absolute amounts that were found. The remarkable fact is that a low exposure to prothioconazole does not necessarily lead to a high conversion to prothioconazole-desthio, but it is obvious that the highest percentage of conversion always occurs where very low absolute amounts of prothioconazole and prothioconazole-desthio are found.

Taking into account all these results the following considerations are applied for a conservative risk assessment of occupational exposure to prothioconazole-desthio:

- For estimating potential dermal exposure to prothioconazole-desthio the highest single figures (*i.e.* from different operators) for the below are taken and added up:
  - outer clothing
  - undergarments
  - cap
  - protective gloves
  - hand washings
- For estimating actual dermal exposure the highest single figures (*i.e.* from different operators) for the below are taken and added up:
  - undergarments
  - cap
  - hand washings

For estimating inhalation exposure to prothioconazole-desthio the normalised values of 0.17 µg/kg as for mixing/loading and application, each, are taken and added up. This is considered to be an adequate conservative approach because the figures represent the highest values for inhalation exposure when one sampling device was used for each task throughout all work cycles, *e.g.* CP 7.2.1.2/01 [M-040604-01-1] operator A3. This procedure ensures that the inhalation value is not merely a theoretical sum of values which are all <LOQ and are simply added up as it is the case *e.g.* for the highest figures of 0.35 µg/kg for mixing/loading and application. Here, per task and work cycle always a new sampling device was used with results <LOQ, each (refer to Table CP 7.2.1.2/01-4)

Table CP 7.2.1.2-8 Collective overview of study parameters from all three operator studies: specific exposure figures for prothioconazole-desthio during downward directed boom application

Exposure	Prothioconazole-desthio (mg/kg a.s.)
Potential dermal exposure[1]	0.251
Actual dermal exposure[2]	0.013

Inhalation exposure[3]	0.00034

1. total amount of active substance which can be found on the operator if tractors were equipped with a cabin.
2. clothing scenario of one layer of clothing and sturdy footwear.
3. Gloves worn during mixing/loading, in addition gloves also worn when contaminated surfaces are handled (*e.g.* folding the boom)

These data represent a conservative approach which can be considered reasonable with regard to the toxicological properties of prothioconazole-desthio:

- maximum figures are taken from different operators,
- head exposure – though no measurable figures were found – being also included as maximum figure.

In addition, the studies were conducted in three different years and comprise a reasonable large number of replicates. Hence, these data are considered representative for the specific assessment to prothioconazole-desthio.

With regard to prothioconazole, the study results can also be used for the risk assessment. One possible option is the evaluation of the data in a similar way as for prothioconazole-desthio. However, taking into account the favourable toxicological profile of the active substance such a very conservative approach is not deemed to be necessary. Therefore, the 75th percentile as well as the geometric mean of the potential and actual exposure values are presented. Calculation of these values is done by first adding up the corresponding figures of the different sampling devices (potential body exposure: outer clothing, undergarments, cap; actual body exposure: undergarments, cap; potential hand exposure: protective gloves, hand washings; actual hand exposure: hand washings) and subsequent calculation of the corresponding point estimate.

Table CP 7.2.1.2-9 Collective overview of study parameters from all three operator studies: specific exposure figures for prothioconazole-desthio downward directed boom application

Exposure	Prothioconazole (mg/kg a.s.)	
	Geometric mean	75th percentile
Potential dermal exposure: body	0.060	0.116
Potential dermal exposure: hands[1]	0.628	1.06
Actual dermal exposure: body	0.007	0.010
Actual dermal exposure: hands	0.001	0.002
Inhalation exposure: mix/load	0.00012	0.00022
Inhalation exposure: application	0.00013	0.00031

1. Potential hand exposure can't be used for generic exposure figures since several farmers rinsed the gloves under water before taking them off.

In addition, the study results can also be used in a generic manner as opposed to the special issue of prothioconazole-desthio. The study data lend itself to be used in exposure assessments for formulations containing additional active substances apart from prothioconazole. To serve for this purpose the data are expressed as "prothioconazole equivalents", *i.e.* the results of prothioconazole-desthio are converted to "prothioconazole results" (by multiplication with 1.103, derived from the molar ratio) and added to the results of prothioconazole, giving "prothioconazole-equivalents".

Table CP 7.2.1.2-10: Collective overview of study parameters from all three operator studies: normalised 'prothioconazole-equivalents'

Assigned operator ID	Normalised dermal exposure (mg/kg a.s.)									Reference
	Potential dermal exposure			Actual dermal exposure			Inhalation exposure			
	Body	Hands	Total	Body	Hands	Total	M/L	A	Total	
A1	0.103	0.426	0.529	0.014	0.001	0.015	0.74	0.74	1.48	CP 7.2.1.2/01 M-040604-01-1
B1	0.049	1.24	0.129	0.014	0.001	0.014	0.82	0.73	1.55	
C1	0.056	0.887	0.944	0.014	0.001	0.014	0.73	0.73	1.46	
B2	0.049	0.431	0.48	0.014	0.001	0.014	0.82	0.73	1.55	

C2	0.14	3.61	3.75	0.014	0.002	0.016	0.73	0.73	1.46	
A3	0.135	2.79	2.92	0.013	0.002	0.015	0.36	0.36	0.72	
C3	0.066	5.48	5.54	0.013	0.003	0.016	0.36	0.36	0.72	
B3	0.052	3.59	3.65	0.013	0.003	0.017	0.36	0.36	0.72	
A	0.152	0.754	0.906	0.004	0.019	0.023	0.13	0.08	0.22	CP 7.2.1.2/02 M-285798-01-1
B	0.074	1.02	1.09	0.014	0.003	0.017	0.19	0.19	0.38	
C	0.333	1.08	1.33	0.016	0.002	0.018	0.21	0.13	0.34	
D	0.02	0.108	0.128	0.004	0.001	0.005	0.06	0.06	0.13	
E	0.05	0.305	0.355	0.01	0.002	0.012	0.15	0.15	0.29	
A	0.09	0.833	0.923	0.015	0.004	0.019	0.32	0.32	0.64	CP 7.2.1.2/03 M-286545-01-1
B	0.035	0.624	0.659	0.004	0.002	0.007	0.12	0.31	0.42	
C	0.177	0.099	0.275	0.002	0.001	0.003	0.05	0.05	0.09	
D	0.021	0.047	0.069	0.005	0.001	0.005	0.12	0.12	0.25	
E	0.216	0.614	0.83	0.01	0.003	0.013	0.26	0.44	0.70	
F	0.06	0.886	0.947	0.008	0.002	0.009	0.21	0.32	0.52	
H	0.103	0.426	0.529	0.014	0.001	0.015	0.74	0.74	1.48	

The 75th percentile as well as the geometric mean of the exposure values are presented below and these data can be used to assess potential exposure and actual dermal exposure and inhalation exposure for any additional active substance concurrently applied with prothioconazole.

Table CP 7.2.1.2-11 Collective overview of study parameters from all three operator studies: generic exposure figures for actives applied concurrently with prothioconazole

Exposure	Prothioconazole (mg/kg a.s.)	
	Geometric mean	75th percentile
Potential dermal exposure: body	0.080	0.143
Potential dermal exposure: hands[1]	0.671	1.12
Actual dermal exposure: body	0.009	0.014
Actual dermal exposure: hands	0.002	0.003
Inhalation exposure: mix/load	0.00025	0.00045
Inhalation exposure: application	0.00026	0.00051

1. Potential hand exposure can't be used for generic exposure figures since several farmers rinsed the gloves under water before taking them off.

Assessment and conclusion by applicant:

Assessment: This study is deemed acceptable and meets the requirements in 284/2013.

Conclusion: Collectively, estimated operator exposure to prothioconazole as well as to the metabolite, prothioconazole-desthio, and spiroxamine can be estimated using data from the mixing/loading and application specific exposure studies. The results of these studies are considered to represent higher tiered data for the given application scenario than the EFSA model. The data have been used to model exposure to prothioconazole, prothioconazole-desthio and spiroxamine following application of Prothioconazole + Spiroxamine EC460 (data presented CP 7.2.1.2)

CP 7.2.2 Bystander and resident exposure

A summary of the critical GAPs under consideration is presented in Table CP 7.2-1.

A summary of the estimated exposure of bystanders and residents to spiroxamine as a result of the critical exposure scenario is shown in Table CP 7.2.2-. For bystander exposure, each exposure pathway (spray drift, vapour, surface deposit, entry into treated crops) is considered separately, whereas for resident exposure, total systemic exposure for each age group is the sum of the mean values of each exposure pathway.

Table CP 7.2.2-1: Summary of estimations of bystander and resident exposure in relation to the respective reference values using the spray drift measurement data and the EFSA model

Parameter	Adult resident		Child resident		Adult resident		Child resident	
	Systemic exp. (mg/kg bw/d)	%AOEL	Systemic exp. (mg/kg bw/d)	%AOEL	Systemic exp. (mg/kg bw/d)	%AOEL	Systemic exp. (mg/kg bw/d)	%AOEL
	Prothioconazole				Prothioconazole-desthio			
Spray drift (measurement of exposure)	0.00111	0.56	0.00379	1.90	0.000660	6.60	0.00097	9.75
Vapour (EFSA default)	0.0002	0.12	0.0011	0.54	0.00023	2.30	0.0011	10.70
Surface deposits (EFSA default)	0.0011	0.55	0.0026	1.32	0.0011	11.04	0.0026	26.39
Entry into treated crops (EFSA+ refined DFR value)	0.0024	1.20	0.0042	2.10	0.0010	10.00	0.0018	18.00
Sum of all pathways	0.0048	2.42	0.01170	5.85	0.00299	29.94	0.00648	32.42
Parameter	**Adult bystander**		**Child bystander**		**Adult resident**		**Child resident**	
Parameter	**Systemic exp. (mg/kg bw/d)**	**%AAOEL**	**Systemic exp. (mg/kg bw/d)**	**%AAOEL**	**Systemic exp. (mg/kg bw/d)**	**%AAOEL**	**Systemic exp. (mg/kg bw/d)**	**%AAOEL**
	Spiroxamine							
Spray drift (measurement of exposure)	0.00089	1.46	0.00242	3.97	0.000864	5.76	0.00232	15.46
Vapour (EFSA default)	0.0002	0.38	0.0011	1.75	0.00023	1.53	0.0011	7.13
Surface deposits (EFSA default)	0.0029	4.79	0.0071	11.68	0.0010	6.46	0.0024	15.94
Entry into treated crops (EFSA+ DFR value)	0.0021	3.44	0.0039	6.39	0.0021	14.00	0.0039	26.00
Sum of all pathways	-	-	-	-	0.00416	27.75	0.0154	76.86

Conclusion

The algorithms used to estimate bystander (spiroxamine only) resident (prothioconazole (prothioconazole-desthio) and spiroxamine) exposures are embedded in the model and use data from the 95th and 75th percentiles, respectively.

According to the EFSA model calculations, when actual dermal exposure generated data and DFR data are used to refine the spray drift and entry into treated crops scenario along with default EFSA values for vapour and surface deposit, it can be concluded that the risk for bystanders and residents exposed to the active ingredients, prothioconazole (its metabolite, prothioconazole-desthio) in Prothioconazole + Spiroxamine EC 460 (160+300 g/L) is acceptable following application to field (low) crops, using a standard 2 meter buffer. This modelled scenario confirms that drift technology is required to achieve acceptable exposure following re-entry into treated crops, using the EFSA to estimate this route of exposure.

Therefore is can be concluded that the risk for bystander and resident exposed to the active ingredients in Prothioconazole + Spiroxamine EC 460 (160+300 g/L) is acceptable following application to field (low) crops. This has no labelling implications.

CP 7.2.2.1 Estimation of bystander and resident exposure

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

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

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

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

Table CP 7.2.2.1-1 Summary of estimations of bystander exposure in relation to the AAOEL using the EFSA model, tier 1 assessment (*i.e.* no drift reduction considered)

Model data	Age group	Absorbed dose (mg/kg bw/d)				% AAOEL	Reference
		Spray drift	Vapour	Surface deposits	Entry into treated crops		
Tractor-mounted boom sprayer application outdoors to cereals *Application rate: 1.25 L product/ha, 14 day spray interval between applications*							
EFSA model • 0.375 kg SPX/ha • 10 kg[1], 60 kg[2]	Child	0.0505	0.0011	0.0071	0.0240	1.75 – 82.76	Table CP 7.2.1.1-10 (input parameter) Table CP 7.2.2.1-2 (exposure estimate)
	Adult	0.0137	0.0002	0.0029	0.0133	0.38 – 22.42%	

Absorbed dose values presented in **bold** exceed the assigned AAOEL

1 Default child body weight

2 Default adult body weight

Table CP 7.2.2.1-2: Bystander exposure results for field application Prothioconazole + Spiroxamine EC 460 (160+300 g/L) to cereals (0.375 kg SPX/ha) – tractor-mounted boom sprayer application using the EFSA model, no refinement

Bystander - child	Spray drift (95th percentile) (mg/kg bw/day)	0.0505	% of RVAAS	82.76%
	Vapour (95th percentile) (mg/kg bw/day)	0.0011	% of RVAAS	1.75%
	Surface deposits (95th percentile) (mg/kg bw/day)	0.0071	% of RVAAS	11.68%
	Entry into treated crops (95th percentile) (mg/kg bw/day)	0.0240	% of RVAAS	39.34%
Bystander - adult	Spray drift (95th percentile) (mg/kg bw/day)	0.0137	% of RVAAS	22.42%
	Vapour (95th percentile) (mg/kg bw/day)	0.0002	% of RVAAS	0.38%
	Surface deposits (95th percentile) (mg/kg bw/day)	0.0029	% of RVAAS	4.79%
	Entry into treated crops (95th percentile) (mg/kg bw/day)	0.0133	% of RVAAS	21.85%

Table CP 7.2.2.1-3 Summary of estimations of resident exposure in relation to the AOEL using the EFSA model, tier I assessment (*i.e.* no drift reduction considered)

Model data	Age group	Absorbed dose (mg/kg bw/d)					% AOEL	Reference
		Spray drift	Vapour	Surface deposits	Entry into treated crops	All pathways (mean)		
Tractor-mounted boom sprayer application outdoors to cereals *Application rate: 1.25 L product/ha, 14 day spray interval between applications*								
EFSA model • 0.2 kg PTZ/ha • 10 kg[1], 60 kg[2]	Child	0.0139	0.0011	0.0006	0.0273	0.0243	12.16	Table CP 7.2.1.1-4 (input parameter)
	Adult	0.0026	0.0002	0.0003	0.0152	0.0140	6.98	Table CP 7.2.2.1-4 (exposure estimate)
EFSA model • 0.2 kg PTZ desthio/ha • 10 kg[1], 60 kg[2]	Child	0.0252	0.0011	0.0026	0.0273	0.0387	**387.11**	Table CP 7.2.1.1-7 (input parameter)
	Adult	0.0060	0.0002	0.0011	0.0152	0.0160	160.19	Table CP 7.2.2.1-5 (exposure estimate)
EFSA model • 0.375 kg SPX/ha • 10 kg[1], 60 kg[2]	Child	0.0222	0.0011	0.0024	0.0240	0.0342	**227.96**	Table CP 7.2.1.1-10 (input parameter)
	Adult	0.0053	0.0002	0.0010	0.0133	0.0141	93.94	Table CP 7.2.2.1-6 (exposure estimate)

Absorbed dose values presented in **bold** exceed the assigned AOEL

1 Default child body weight

2 Default adult body weight

Table CP 7.2.2.1-4 Resident exposure results for field application Prothioconazole + Spiroxamine EC 460 (160+300 g/L) to cereals (0.20 kg PTZ/ha) – tractor-mounted boom sprayer application using the EFSA model, no refinement

Resident - child	Spray drift (75th percentile) (mg/kg bw/day)	0.0139	% of RVNAS	6.94%
	Vapour (75th percentile) (mg/kg bw/day)	0.0011	% of RVNAS	0.54%
	Surface deposits (75th percentile) (mg/kg bw/day)	0.0006	% of RVNAS	0.31%
	Entry into treated crops (75th percentile) (mg/kg bw/day)	0.0273	% of RVNAS	13.67%
	All pathways (mean) mg/kg bw/day	0.0243	% of RVNAS	12.16%
Resident - adult	Spray drift (75th percentile) (mg/kg bw/day)	0.0026	% of RVNAS	1.31%
	Vapour (75th percentile) (mg/kg bw/day)	0.0002	% of RVNAS	0.12%
	Surface deposits (75th percentile) (mg/kg bw/day)	0.0003	% of RVNAS	0.13%
	Entry into treated crops (75th percentile) (mg/kg bw/day)	0.0152	% of RVNAS	7.59%
	All pathways (mean) mg/kg bw/day	0.0140	% of RVNAS	6.98%

Table CP 7.2.2.1-5 Resident exposure results for field application Prothioconazole + Spiroxamine EC 460 (160+300 g/L) to cereals (0.20 kg PTZ desthio/ha) – tractor-mounted boom sprayer application using the EFSA model, no refinement

Resident - child	Spray drift (75th percentile) (mg/kg bw/day)	0.0252	% of RVNAS	252.49%
	Vapour (75th percentile) (mg/kg bw/day)	0.0011	% of RVNAS	10.70%
	Surface deposits (75th percentile) (mg/kg bw/day)	0.0026	% of RVNAS	26.39%
	Entry into treated crops (75th percentile) (mg/kg bw/day)	0.0273	% of RVNAS	273.41%
	All pathways (mean) mg/kg bw/day	0.0387	% of RVNAS	387.11%
Resident - adult	Spray drift (75th percentile) (mg/kg bw/day)	0.0060	% of RVNAS	60.41%
	Vapour (75th percentile) (mg/kg bw/day)	0.0002	% of RVNAS	2.30%
	Surface deposits (75th percentile) (mg/kg bw/day)	0.0011	% of RVNAS	11.04%
	Entry into treated crops (75th percentile) (mg/kg bw/day)	0.0152	% of RVNAS	151.90%
	All pathways (mean) mg/kg bw/day	0.0160	% of RVNAS	160.19%

Table CP 7.2.2.1-6 Resident exposure results for field application Prothioconazole + Spiroxamine EC 460 (160+300 g/L) to cereals (0.375 kg SPX/ha) – tractor-mounted boom sprayer application using the EFSA model, no refinement

Resident - child	Spray drift (75th percentile) (mg/kg bw/day)	0.0222	% of RVNAS	148.03%
	Vapour (75th percentile) (mg/kg bw/day)	0.0011	% of RVNAS	7.13%
	Surface deposits (75th percentile) (mg/kg bw/day)	0.0024	% of RVNAS	15.94%
	Entry into treated crops (75th percentile) (mg/kg bw/day)	0.0240	% of RVNAS	159.97%
	All pathways (mean) mg/kg bw/day	0.0342	% of RVNAS	227.96%
Resident - adult	Spray drift (75th percentile) (mg/kg bw/day)	0.0053	% of RVNAS	35.37%
	Vapour (75th percentile) (mg/kg bw/day)	0.0002	% of RVNAS	1.53%
	Surface deposits (75th percentile) (mg/kg bw/day)	0.0010	% of RVNAS	6.46%
	Entry into treated crops (75th percentile) (mg/kg bw/day)	0.0133	% of RVNAS	88.87%
	All pathways (mean) mg/kg bw/day	0.0141	% of RVNAS	93.94%

Taking the approach presented in Table CP 7.2.2.1-2 and Table CP 7.2.2.1-6, refinement to the worst case scenario is required for prothioconazole-desthio. Refinement of exposure to prothioconazole-desthio is estimated assuming 100% conversion for inhalation exposure and 50% conversion for dermal exposure. In addition, no correction with respect to the molar ratio is made. The 50% prothioconazole to prothioconazole-desthio conversion results in 50% reduction in the initial spray drift, surface deposit (dermal) and entry into treated crops (dermal) initially presented in Table CP 7.2.2.1-7 and Table CP 7.2.2.1-9, without correction to the other parameters (vapour, surface deposits (hand to mouth, object to mouth). For consistency, vehicle-mounted drift reduction nozzles and a 10 m buffer strip have been considered throughout

Table CP 7.2.2.1-7 Summary of estimations of bystander exposure in relation to the AAOEL using the EFSA model, tier II assessment

Model data	Age group	Absorbed dose (mg/kg bw/d)				% AAOEL	Reference
		Spray drift	Vapour	Surface deposits	Entry intro treated crops		
Tractor-mounted boom sprayer application outdoors to cereals *Application rate: 1.25 L product/ha, 14 day spray interval between applications*							
EFSA model • 0.375 kg SPX/ha • Vehicle drift reduction nozzles • 10 m buffer strip • 10 kg[1], 60 kg[2]	Child	0.0132	0.0014	0.0008	0.0240	1.31 – 39.34	Table CP 7.2.2.1-8 (input parameter) Table CP 7.2.2.1-9 (exposure estimate)
	Adult	0.0027	0.0002	0.0003	0.0133	0.38 – 21.85	

1 Default child body weight
2 Default adult body weight
3 Consideration of 50% conversion of prothioconazole to prothioconazole desthio

Table CP 7.2.2.1-8 Input parameters for the EFSA model for the active substance spiroxamine when applied to cereals (field), tier II assessment for bystander and resident exposure

Substance name	Spiroxamine (SPX)	
Product name	PTZ + SPX EC 460 (160+300 g/L)	
Reference value non acutely toxic active substance (RVNAS)	0.015	mg/kg bw/day
Reference value acutely toxic active substance (RVAAS)	0.0614	mg/kg bw/day
Crop type	Cereals	
Substance properties		
Formulation type	Soluble concentrates, emulsifiable concentrate, etc.	
Miniumum volume water for application (liquids)	100	L/ha
Maximum application rate of active substance	0.375	kg a.s. /ha
50% Dissipation Time DT_{50}	30	days
Initial Dislodgeable Foliar Residue	3	$\mu g/cm^2$ of foliage/kg a.s. applied/ha
Dermal absorption of product	9.87%	
Dermal absorption of in-use dilution	22.00%	
Oral absorption of active substance	61.00%	
Inhalation absorption of active substance	100.00%	
Vapour pressure of active substance	low volatile substances having a vapour pressure of <5*10-3Pa	
Scenario		
Indoor or Outdoor application	Outdoor	
Application method	Downward spraying	
Application equipment	Vehicle-mounted-Drift Reduction	
Buffer strip	10	m
Number of applications	2	
Interval between multiple applications	14	days
Season (upward spraying orchards only)	not relevant	

Table CP 7.2.2.1-9 Bystander exposure results for field application Prothioconazole + Spiroxamine EC 460 (160+300 g/L) to cereals (0.375 kg SPX/ha) – tractor-mounted boom sprayer application using the EFSA model with refinement considerations

Bystander - child	Spray drift (95th percentile) (mg/kg bw/day)	0.0132	% of RVAAS	21.71%
	Vapour (95th percentile) (mg/kg bw/day)	0.0011	% of RVAAS	1.75%
	Surface deposits (95th percentile) (mg/kg bw/day)	0.0008	% of RVAAS	1.31%
	Entry into treated crops (95th percentile) (mg/kg bw/day)	0.0240	% of RVAAS	39.34%
Bystander - adult	Spray drift (95th percentile) (mg/kg bw/day)	0.0027	% of RVAAS	4.42%
	Vapour (95th percentile) (mg/kg bw/day)	0.0002	% of RVAAS	0.38%
	Surface deposits (95th percentile) (mg/kg bw/day)	0.0003	% of RVAAS	0.54%
	Entry into treated crops (95th percentile) (mg/kg bw/day)	0.0133	% of RVAAS	21.85%

Table CP 7.2.2.1-10 Summary of estimations of resident exposure in relation to the AAOEL using the EFSA model, tier II assessment

Model data	Age group	Absorbed dose (mg/kg bw/d)					% AOEL	Reference
		Spray drift	Vapour	Surface deposits	Entry into treated crops	All pathways (mean)		
Tractor-mounted boom sprayer application outdoors to cereals *Application rate: 1.25 L product/ha, 14 day spray interval between applications*								
EFSA model • 0.2 kg PTZ/ha • Vehicle drift reduction nozzles • 10 m buffer strip • 10 kg[1] • 10 kg[1], 60 kg[2]	Child	0.0069	0.0011	0.0003	0.00273	0.0270	13.49	Table CP 7.2.2.1-11 (input parameter) Table CP 7.2.2.1-12 (exposure estimate)
	Adult	0.013	0.0002	0.0001	0.0152	0.0131	6.57	
EFSA model • 0.2 kg PTZ desthio/ha[3] • Vehicle drift reduction nozzles • 10 m buffer strip • 10 kg[1], 60 kg[2]	Child	0.0035	0.0011	0.0002	0.0137	0.0140	**140.20**	Table CP 7.2.2.1-13 (input parameter) Table CP 7.2.2.1-14 (exposure estimate)
	Adult	0.0007	0.0002	0.0001	0.0076	0.0067	66.88	
EFSA model • 0.375 kg SPX/ha • Vehicle drift reduction nozzles • 10 m buffer strip • 10 kg[1], 60 kg[2]	Child	0.0061	0.0011	0.0003	0.0240	0.0238	**158.80**	Table CP 7.2.2.1-8 (input parameter) Table CP 7.2.2.1-15 (exposure estimate)
	Adult	0.0012	0.0002	0.0001	0.0133	0.0116	77.11	

Absorbed dose values presented in **bold** exceed the assigned AOEL

1 Default child body weight

2 Default adult body weight

3 Consideration of 50% conversion of prothioconazole to prothioconazole desthio

Table CP 7.2.2.11 Input parameters for the EFSA model for the active substance prothioconazole when applied to cereals (field), tier II assessment for bystander and resident exposure

Substance name	Prothioconazole (PTZ)	
Product name	PTZ + SPX EC 460 (160+300 g/L)	
Reference value non acutely toxic active substance (RVNAS)	0.2	mg/kg bw/day
Reference value acutely toxic active substance (RVAAS)		mg/kg bw/day
Crop type	Cereals	
Substance properties		
Formulation type	Soluble concentrates, emulsifiables concentrate, etc.	
Miniumum volume water for application (liquids)	100	L/ha
Maximum application rate of active substance	0.2	kg a.s. /ha
50% Dissipation Time DT_{50}	30	days
Initial Dislodgeable Foliar Residue	3	µg/cm² of foliage/kg a.s. applied/ha
Dermal absorption of product	25.00%	
Dermal absorption of in-use dilution	47.00%	
Oral absorption of active substance	100.00%	
Inhalation absorption of active substance	100.00%	
Vapour pressure of active substance	low volatile substances having a vapour pressure of <5*10-3Pa	
Scenario		
Indoor or Outdoor application	Outdoor	
Application method	Downward spraying	
Application equipment	Vehicle-mounted-Drift Reduction	
Buffer strip	10	m
Number of applications	2	
Interval between multiple applications	14	days
Season (upward spraying orchards only)	not relevant	

Table CP 7.2.2.1-12 Resident exposure results for field application Prothioconazole + Spiroxamine EC 460 (160+300 g/L) to cereals (0.20 kg PTZ/ha) – tractor-mounted boom sprayer application using the EFSA model with refinement considerations

Resident - child	Spray drift (75th percentile) (mg/kg bw/day)	0.0069	% of RVNAS	3.47%
	Vapour (75th percentile) (mg/kg bw/day)	0.0011	% of RVNAS	0.54%
	Surface deposits (75th percentile) (mg/kg bw/day)	0.0003	% of RVNAS	0.15%
	Entry into treated crops (75th percentile) (mg/kg bw/day)	0.0273	% of RVNAS	13.67%
	All pathways (mean) mg/kg bw/day	0.0270	% of RVNAS	13.49%
Resident - adult	Spray drift (75th percentile) (mg/kg bw/day)	0.0013	% of RVNAS	0.66%
	Vapour (75th percentile) (mg/kg bw/day)	0.0002	% of RVNAS	0.12%
	Surface deposits (75th percentile) (mg/kg bw/day)	0.0001	% of RVNAS	0.06%
	Entry into treated crops (75th percentile) (mg/kg bw/day)	0.0152	% of RVNAS	7.59%
	All pathways (mean) mg/kg bw/day	0.0131	% of RVNAS	6.57%

Table CP 7.2.2.13 Input parameters for the EFSA model for the metabolite prothioconazole-desthio when applied to cereals (field), tier II assessment for bystander and resident exposure

Substance name	Prothioconazole desthio (PTZ desthio)	
Product name	PTZ + SPX EC 460 (160+300 g/L)	
Reference value non acutely toxic active substance (RVNAS)	0.01	mg/kg bw/day
Reference value acutely toxic active substance (RVAAS)		mg/kg bw/day
Crop type	Cereals	
Substance properties		
Formulation type	Soluble concentrates, emulsifiable concentrate, etc.	
Miniumum volume water for application (liquids)	100	L/ha
Maximum application rate of active substance	0.2	kg a.s. /ha
50% Dissipation Time DT_{50}	30	days
Initial Dislodgeable Foliar Residue	3	$\mu g/cm^2$ of foliage/kg a.s. applied/ha
Dermal absorption of product	25.00%	
Dermal absorption of in-use dilution	47.00%	
Oral absorption of active substance	100.00%	
Inhalation absorption of active substance	100.00%	
Vapour pressure of active substance	low volatile substances having a vapour pressure of <5*10-3Pa	
Scenario		
Indoor or Outdoor application	Outdoor	
Application method	Downward spraying	
Application equipment	Vehicle-mounted-Drift Reduction	
Buffer strip	10	m
Number of applications	2	
Interval between multiple applications	14	days
Season (upward spraying orchards only)	not relevant	

Table CP 7.2.2.1-14 Resident exposure results for field application Prothioconazole + Spiroxamine EC 460 (160+300 g/L) to cereals (0.20 kg PTZ desthio/ha) – tractor-mounted boom sprayer application using the EFSA model with refinement considerations

Resident - child	Spray drift (75th percentile) (mg/kg bw/day)	0.0035	% of RVNAS	34.68%
	Vapour (75th percentile) (mg/kg bw/day)	0.0011	% of RVNAS	10.70%
	Surface deposits (75th percentile) (mg/kg bw/day)	0.0002	% of RVNAS	1.69%
	Entry into treated crops (75th percentile) (mg/kg bw/day)	0.0137	% of RVNAS	136.71%
	All pathways (mean) mg/kg bw/day	0.0140	% of RVNAS	140.20%
Resident - adult	Spray drift (75th percentile) (mg/kg bw/day)	0.0007	% of RVNAS	6.35%
	Vapour (75th percentile) (mg/kg bw/day)	0.0002	% of RVNAS	2.30%
	Surface deposits (75th percentile) (mg/kg bw/day)	0.0001	% of RVNAS	0.64%
	Entry into treated crops (75th percentile) (mg/kg bw/day)	0.0076	% of RVNAS	75.95%
	All pathways (mean) mg/kg bw/day	0.0067	% of RVNAS	66.88%

Dermal exposure from the 'spray drift', 'surface deposits' and 'entry into treated crops' adjust in EFSA model to take by 50% conversion of prothioconazole to prothioconazole-desthio, with EFSA model input parameters for prothioconazole remaining unchanged. Inhalation exposure was not corrected.

Table CP 7.2.2.1-15 Resident exposure results for field application Prothioconazole + Spiroxamine EC 460 (160+300 g/L) to cereals (0.375 kg SPX/ha) – tractor-mounted boom sprayer application using the EFSA model with refinement considerations

Resident - child	Spray drift (75th percentile) (mg/kg bw/day)	0.0061	% of RVNAS	40.67%
	Vapour (75th percentile) (mg/kg bw/day)	0.0011	% of RVNAS	7.13%
	Surface deposits (75th percentile) (mg/kg bw/day)	0.0003	% of RVNAS	1.85%
	Entry into treated crops (75th percentile) (mg/kg bw/day)	0.0240	% of RVNAS	159.97%
	All pathways (mean) mg/kg bw/day	0.0238	% of RVNAS	158.80%
Resident - adult	Spray drift (75th percentile) (mg/kg bw/day)	0.0012	% of RVNAS	7.68%
	Vapour (75th percentile) (mg/kg bw/day)	0.0002	% of RVNAS	1.53%
	Surface deposits (75th percentile) (mg/kg bw/day)	0.0001	% of RVNAS	0.75%
	Entry into treated crops (75th percentile) (mg/kg bw/day)	0.0133	% of RVNAS	88.87%
	All pathways (mean) mg/kg bw/day	0.0116	% of RVNAS	77.11%

Taking the approach presented in Table CP 7.2.2.1-10, refinement for the active spiroxamine and the metabolite, prothioconazole-desthio is required when vehicle-mounted drift reduction nozzles and a 10 m buffer strip have been considered. It is acknowledge that the evaluation of exposure for the entry into treated crops directly after application and using high end EFSA default values results in a very conservative approach. In addition, a potential accumulation of residues after repeated application is considered in this calculation although in the context of current resistance prevention strategies, the product is used within spray programmes, *i.e.* alternating with products of other chemical groups with different modes of actions rendering this scenario to be unlikely in reality. Nevertheless consecutive sprays are considered in this evaluation as a worst possible case. Therefore, there is a need to refine the risk assessment with available bystander/resident measurement exposure.

Conclusion

The algorithms used to estimate bystander (spiroxamine only) resident (prothioconazole (prothioconazole-desthio) and spiroxamine) exposures are embedded in the model and use data from the 95th and 75th percentiles, respectively.

According to the EFSA model calculations it can be concluded that the risk for bystanders exposed to the active ingredient, spiroxamine is acceptable. In the absence of an AAOEL established for prothioconazole or its metabolite, prothioconazole-desthio, bystander modelling has not been assessed.

For residents exposed to the active ingredients, prothioconazole (its metabolite, prothioconazole-desthio) in Prothioconazole + Spiroxamine EC 460 (160+300 g/L), exceedance of the AOEL for child residents for both prothioconazole-desthio and spiroxamine following application to field (low) crops when vehicle-mounted drift reduction nozzles and a 10 m buffer strip have been considered. Therefore, there is a need to refine the risk assessment with available bystander/resident measurement exposure.

CP 7.2.2.2 Measurement of bystander and resident exposure

Two bystander/resident exposure studies are available which were previously submitted for prothioconazole renewal. In the first study, exposure of bystanders / residents to spiroxamine and prothioconazole from spray applications with Input® in cereals using standard spray nozzles dermal and inhalation exposure to prothioconazole and prothioconazole-desthio due to spray drift was measured. In the second study, dermal exposure due to spray drift of bystanders / residents to prothioconazole and its main metabolite prothioconazole-desthio from tractor mounted/trailed boom sprayers with Aviator Xpro EC 225 in cereals was measured.

A low number of replicates were used in both studies for mannequins positioned 2 meters from the sprayed area. In the first evaluated study using the 'Input' formulation there were three mannequins representing adult bystanders/residents and three mannequins representing child bystanders/residents monitored at a distance of 2 meters from the sprayed area. In the second evaluated study using the 'Aviator Xpro EC 225' formulation there were five mannequins representing adult bystanders/residents and five mannequins representing child bystanders/residents monitored at a distance of 2 meters from the sprayed area. Furthermore, comparison of the spray application parameters from both studies with the parameters in the BREAM calculator (refer to CP 7.2.2.2/01, Materials and methods, Section B and CP 7.2.2.2/02, Materials and methods, Section B) indicates that the drift would be lower in the studies compared to the scenario taken from BREAM. The studies provided were undertaken using a lower operating pressure, lower forward speed and lower average wind speed during application (CP 7.2.2.2/01 [M-510333-01-1]) compared to the BREAM scenario. During stop-the-clock the applicant provided additional information from a wind tunnel experiment.

The objective of the wind tunnel study was to establish the difference in drift relevant to bystander exposure, between a Teejet XR110 03 VP nozzle at 1.3 bar and 9 km/h and a conventional flat fan 110 03 nozzle (Hypro) at 3.0 bar by measurements of Droplet size distribution using laser diffraction and wind tunnel measurements of spray drift. The results indicate that a validation factor of 1.18 could be applied to make a comparison between BREAM and both studies possible.

The applicant proposes to correct the dermal exposure residues from both studies with the proposed factor of 1.18. A summary of this wind tunnel experiment is presented in this chapter. It should be noted that the second study (CP 7.2.2.2/02) reported to have a high variability in the wind speed which is reflected in a high variability in the exposure data. More specifically, the amended study report informs us that: 'The measured mean wind speed during the entire spray duration was at 3.8 m/s. However, when the tractor was passing the first dummy pair (adult A1 and child a1) the wind speed was with 2 m/s – 2.5 m/s at the lower range of observed wind speeds. When passing the last dummies (adult A5 and child a5) approximately 30

seconds later, the wind speeds was, however, in a range of about 5 m/s even up to 6 m/s, and thus significantly higher than at the beginning of the study'.

As such, the RMS concluded that it would not be appropriate to substitute the default dermal exposures for bystanders/residents in the EFSA guidance with the 75th percentile value from the submitted studies, especially when considering the difference in application parameters between BREAM and the submitted studies .Therefore, refinement has been undertaken for both bystander and resident exposure using the 95th percentile data from the Input formulation, which mirrors the Prothioconazole + Spiroxamine EC 460, which provides a precautionary estimation of systemic exposure to this cohort *re* spray drift exposure.

With the approach adopted during the previous renewal process, and detailed under CP 7.2.1.2.2, the data have been used in a generic manner as opposed to the special issue of prothioconazole-desthio. The study data lend itself to be used in exposure assessments for formulations containing additional active substances apart from prothioconazole. To serve for this purpose the data are expressed as "prothioconazole equivalents", *i.e.* the results of prothioconazole-desthio are converted to "prothioconazole results" (by multiplication with 1.103, derived from the molar ratio) and added to the results of prothioconazole, giving "prothioconazole-equivalents"

Table CP 7.2.3.2-1: Collective overview of acute dermal exposure values obtained for prothioconazole, prothioconazole-desthio and calculated prothioconazole-equivalents used to refine the bystander/resident exposure assessment

Exposure scenario	Statistic	Prothioconazole (mg/day)	Prothioconazole-desthio (mg/day)	Prothioconazole-equivalents
		(mg/day)	(mg/day)	(mg/day)
Adult	95th percentile	0.142	0.0838	0.234
Child	95th percentile	0.0798	0.0198	0.102

Table CP 7.2.3.2-2: Summary of estimations of bystander and resident exposure in relation to the respective reference values using the spray drift measurement data and the EFSA model

Parameter	Adult resident		Child resident		Adult resident		Child resident	
	Systemic exp. (mg/kg bw/d)	%AOEL	Systemic exp. (mg/kg bw/d)	%AOEL	Systemic exp. (mg/kg bw/d)	%AOEL	Systemic exp. (mg/kg bw/d)	%AOEL
	Prothioconazole				Prothioconazole-desthio			
Spray drift (measurement of exposure)	0.00111	0.56	0.00379	1.90	0.000660	6.60	0.00097	9.75
Vapour (EFSA default)	0.0002	0.12	0.0011	0.54	0.00023	2.30	0.0011	10.70
Surface deposits (EFSA default)	0.0011	0.55	0.0026	1.32	0.0011	11.04	0.0026	26.39
Entry into treated crops (EFSA+ refined DFR value)	0.0024	1.20	0.0042	2.10	0.0010	10.00	0.0018	18.00
Sum of all pathways	0.0048	2.42	0.01170	5.85	0.00299	29.94	0.00648	32.42
Parameter	**Adult bystander**		**Child bystander**		**Adult resident**		**Child resident**	
Parameter	**Systemic exp. (mg/kg bw/d)**	**%AAOEL**	**Systemic exp. (mg/kg bw/d)**	**%AAOEL**	**Systemic exp. (mg/kg bw/d)**	**%AAOEL**	**Systemic exp. (mg/kg bw/d)**	**%AAOEL**
	Spiroxamine							
Spray drift (measurement of exposure)	0.00089	1.46	0.00242	3.97	0.000864	5.76	0.00232	15.46
Vapour (EFSA default)	0.0002	0.38	0.0011	1.75	0.00023	1.53	0.0011	7.13
Surface deposits (EFSA default)	0.0029	4.79	0.0071	11.68	0.0010	6.46	0.0024	15.94
Entry into treated crops (EFSA+ DFR value)	0.0021	3.44	0.0039	6.39	0.0021	14.00	0.0039	26.00
Sum of all pathways	-	-	-	-	0.00416	27.75	0.0154	76.86

Conclusion

The algorithms used to estimate bystander resident exposures are embedded in the model and use data from the 95th and 75th percentiles, respectively.

According to the EFSA model calculations, when actual dermal exposure generated data and DFR data are used to refine the spray drift and entry into treated crops scenario along with default EFSA values for vapour and surface deposit, it can be concluded that the risk for bystanders and residents exposed to the active ingredients, prothioconazole (its metabolite, prothioconazole-desthio) in Prothioconazole + Spiroxamine EC 460 (160+300 g/L) is acceptable following application to field (low) crops, using a standard 2 meter buffer. This modelled scenario confirms that drift technology is required to achieve acceptable exposure following re-entry into treated crops, using the EFSA to estimate this route of exposure.

Therefore is can be concluded that the risk for bystander and resident exposed to the active ingredients in Prothioconazole + Spiroxamine EC 460 (160+300 g/L) is acceptable following application to field (low) crops. This has no labelling implications.

Data Point:	KCP 7.2.2.2/01
Report Author:	[redacted]; [redacted]
Report Year:	2015
Report Title:	Exposure of bystanders / residents to spiroxamine and prothioconazole from spray applications with Input in cereals using standard spray nozzles
Report No:	MR-14/075
Document No:	M-510333-01-1
Guideline(s) followed in study:	OECD Guidance Document for the Conduct of Studies of Occupational Exposure to Pesticides During Agricultural Application, Series on Testing and Assessment No. 9, 1997 Equipment for crop protection - Methods for field measurement of spray drift, ISO 22866:2005(E)
Deviations from current test guideline:	None
Previous evaluation:	yes, evaluated and accepted Prothioconazole RAR (2018)
GLP/Officially recognised testing facilities:	Yes, conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	Yes

Executive summary

The purpose of the study was to determine the dermal and inhalation exposure of bystanders/residents to prothioconazole, its main metabolite prothioconazole-desthio and spiroxamine from spray drift at various distances downwind from the sprayed area during application of 'Input® EC 460' to winter wheat through a field crop boom sprayer. 'Input® EC 460' is formulated as an emulsifiable concentrate comprising the two active ingredients spiroxamine (300 g/L) and prothioconazole (160 g/L). Only exposure to prothioconazole and prothioconazole-desthio has been considered in the RMS study evaluation.

The study was conducted on commercial agricultural land in Germany in May 2012. Headland was selected based on the prevailing wind direction to obtain a track as near as possible to 90° to the prevailing wind direction, representing worst case conditions to persons in the vicinity affected by drift. The crop (wheat) reached a canopy height of about 60 cm and was at a growth stage BBCH 55. The crop was grown on a non-sloped area in an area of more than 10 ha. An area of 100m x 22m was mulched in the centre of the field to allow the positioning of the mannequins in the spray drift zone. Replicates of mannequins were placed at 2, 5 and 8 meters downwind to monitor a range of potential distances where bystanders or residents may be exposed during application.

The area adjacent to the mulched zone was sprayed with one spray swath. The spray application was performed using 'Input® EC 460' with a dose rate of 1.25 L/ha (corresponding to 200 g prothioconazole/ha) using standard spray nozzles (Type: TeeJet XR 110-03). The total area sprayed was 100 m x 28 m = 0.28 ha. The application was conducted with a single spray swath with a 28 meter width, a spray volume of 100 L/ha, a forward speed of 10 km/h and a spray pressure of 1.4 bar. The spray boom was positioned at 1.1 m (0.5 m above canopy height of approximately 0.6 m). Section B (below) provides a comparison of the parameters in the study with the proposed use of 'Aviator Xpro EC 225' and Section C (below) compares the spray application details in the study to the BREAM calculator. Figure B.6.4.2.3- 1 provides the trial layout, while Figure CP 7.2.2.2/01-2-1 provides visual application details for both studies Figure CP 7.2.2.2/01-2 shows a photographic example of the used mannequins and its positioning in the field.

Figure CP 7.2.2.2/01-1: Trial layout

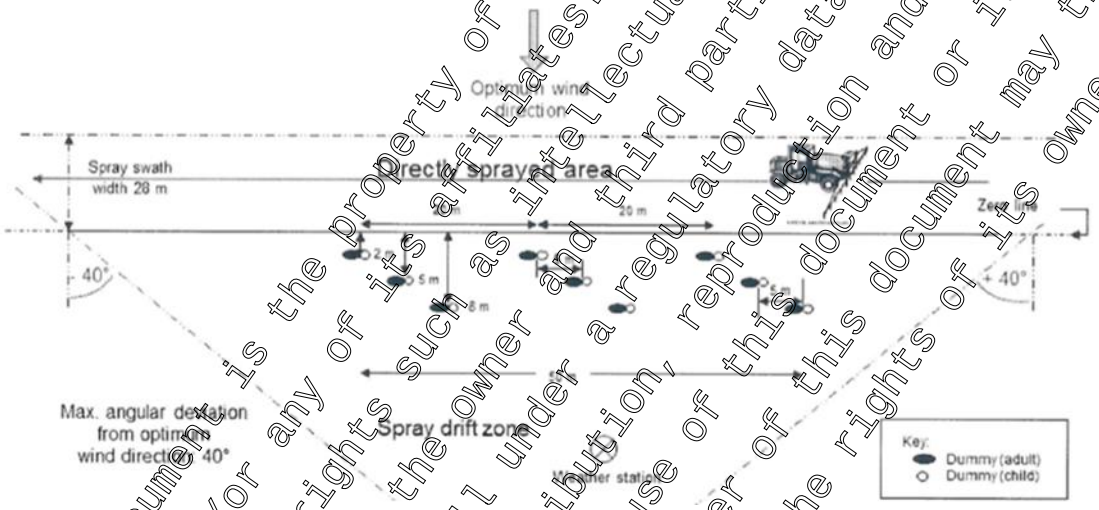
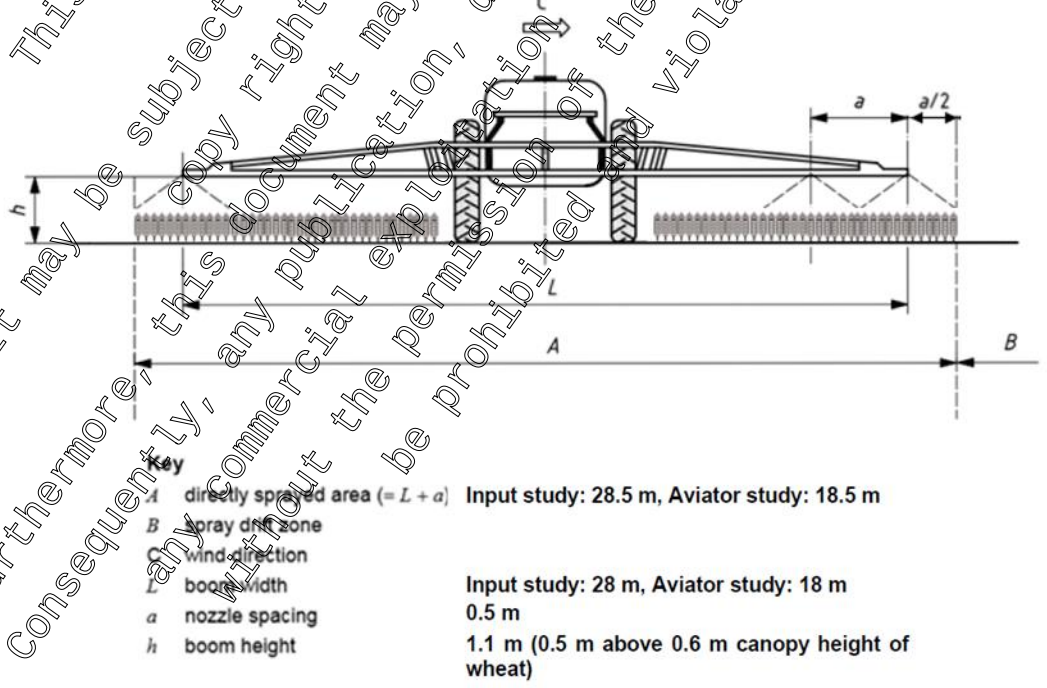


Figure CP 7.2.2.2/01-2: Application details and zero line in both studies



Key		
A	directly sprayed area (= *L* + *a*)	Input study: 28.5 m, Aviator study: 18.5 m
B	spray drift zone	
C	wind direction	
L	boom width	Input study: 28 m, Aviator study: 18 m
a	nozzle spacing	0.5 m
h	boom height	1.1 m (0.5 m above 0.6 m canopy height of wheat)

Figure CP 7.2.2.2/01-3: Photos of bystander mannequins and their positioning in the field



Materials and methods

A. Materials:

1. Test Material:	Prothioconazole + Spiroxamine EC 460 (alternative name: Input® EC 460)	Prothioconazole + Bixafen EC 225 (alternative name: Aviator Xpro EC 225)
Purity:	160 g/L (prothioconazole) 250 g/L (spiroxamine)	160 g/L (prothioconazole) 75 g/L (bixafen)
CAS No.:	178928-70-6 (prothioconazole) 118134-30-8 (spiroxamine)	178928-70-6 (prothioconazole) 581809-46-3 (bixafen)
2. Study conditions:		
Operation time:		
Overall monitoring period:	>5 - >7 h	
Area treated	20 ha	
Amount of a.s. applied:	1.25 L product/ha containing 200 g prothioconazole	1.25 L product/ha containing 187.5 g prothioconazole
Max. total dose	n.a.	2.5 L product/ha (376 g prothioconazole/ha; 188 g bixafen/ha)
Number of applications:	1 (application interval: n/a)	2 (application interval: 14)
Water volume	100 L/ha	100-400 L/ha
3. Equipment used:	Tractor (trailed boom sprayer application	

B. Comparison of spray application parameters used in the study with the BREAM calculator:

	BREAM calculator	**CP 7.2.2.2/01; CP 7.2.2.2/02**
Bystander type	Adult and Child	Adult and Child
Exposure route	Dermal and inhalation	Dermal and inhalation
Nozzle type:	Flat Fan 03110	Standard, TeeJet XR 110-03
No. of nozzles:	48	56
Pressure:	3 bar	1.5 bar
Forward/Driving speed:	12.6 km/h	10 km/h
Boom height:	0.7 m	0.5 m
Crop height:	short	0.6 m
Wind speed:	2.7 m/s	2-5 m/s (average 2.3 m/s) 2.3 m/s*
Spray concentration:	1 g a.s./L spray	2 g prothioconazole/L spray

*RMS PL comment: Wind speeds for both studies were mixed up in the RAR. This is the correct value.

C. Sampling:

The test system consisted of 18 mannequins (9 representing adult bystanders and 9 representing child bystanders). The mannequins intended to represent adults were 1.86 meters high and the mannequins intended to represent children were 1.04 meters high. Adult and child mannequin pairs were positioned 2, 5 and 8 meters from the zero line. For each sampling distance relative to the zero-line, three adult/child mannequin pairs were installed as replicates with a distance of at least 20 m between each pair. The zero line was set at a distance of half the nozzle spacing from the edge of boom. As the nozzles were spaced 0.5 meters apart, the zero line can be calculated as 0.25 meters from the edge of the boom.

Mannequins wore whole body dosimeters made of 100% cotton (except shorts consisting of 65% polyester / 35% cotton) and personal air sampling pumps with IOM-filters as sampling media. IOM samplers were attached to the pump and positioned in the virtual breathing zone of the mannequin. The pumps were calibrated for a flow rate of 2 L/minute before the start of monitoring and their performance was assured after the completion of study. The dermal dosimeters consisted of long underwear [long sleeved shirts and long johns worn below short outer dosimeters (short sleeved T-shirt and shorts)] to represent lightly clothed bystanders/residents. The long sleeves of the shirts were worn to cover the hand therefore an additional hand dosimeter was not required. A ski mask was donned to measure total head exposure.

Wind direction, wind speed, air temperature, relative humidity, dew point and air pressure were monitored under no GLP conditions during the day of application at a frequency of 1 Hz.

On completion of the spraying, mannequins were left in the field for 30 minutes to allow aerosols to settle. Air sampling pumps were then stopped and the flow rate was re-measured and recorded before dosimeter collection started. Cassettes and the filter holders from personal air sampling devices were put separately into pre-labelled vials. The whole body dosimeters were removed from the mannequins. The outer dosimeters (short sleeved T-shirt and shorts) were removed first and both samples were combined and put into pre-labelled HDPE-bottles. The inner dosimeters (long sleeved shirts, long johns) and the ski mask were then collected. These samples were also combined and put into pre-labelled HDPE-bottles. All samples were directly transported to the test facility and placed in frozen storage on receipt. The exact storage period of the frozen samples is not given in the study however it is mentioned that the field recovery samples were stored under the same conditions prior to analysis.

D. Method of analysis:

Residues of prothioconazole and prothioconazole-desthio in the dosimeters for dermal and inhalation exposure were determined by using the analytical method 00598/M001, which has been adapted to the matrices of operator and bystander exposure studies. All extensions of method 00598 were summarised in report MR-13/106 (KCA 4.1.2/42) and consequently the method is now occasionally referred to as Method MR-13/106. All areas of method 00598 which were not originally evaluated for the Annex 1 inclusion are evaluated in the Draft (Renewal) Assessment Report (DAR), Volume 3 – B.5. Thus, all methods referring to MR-13/106, including the method 00598/M001 applied in the current study can also be considered to be fully validated.

E. Field recovery and control samples:

Field recoveries were prepared for the dermal dosimeters and IOM filters and were used to demonstrate the stability of prothioconazole and prothioconazole-desthio in the field samples and check the performance of the analytical method. Dosimeter samples were exposed to ambient conditions at a location near, but isolated from, the test plot to ensure that field recoveries were exposed to the same ambient conditions but away from potential sources of contamination. At the end of the work day, the field recovery samples were collected and handled in the same way as the actual field samples. The process of fortification of dermal dosimeters and IOM filters is not described in the study report. Thus, it is not clear if fortification was performed using the pesticide formulation in the spray matrix (water) as recommended by the OECD guidance document.

The LOQ for both prothioconazole and prothioconazole-desthio was 1 µg/sample for cotton dosimeters and 0.1 µg/sample for IOM filters. The spiking levels for prothioconazole and prothioconazole-desthio

were 10 µg/sample, 100 µg/sample and 1000 µg/sample for dermal dosimeters and 0.1µg/sample and 10 µg/sample for IOM filters. For each spiking level two replicates were performed. The OECD guidance recommends that at least three fortifications should be made at each spiking level and there is no explanation in the study report for the deviation from the guidance. Furthermore, blank control field recovery samples were not carried out.

Field recoveries for samples spiked with prothioconazole are presented in Table CP 7.2.2.2/01-1 and field recoveries for samples spiked with prothioconazole-desthio are presented in Table CP 7.2.2.2/01-2. For field recoveries spiked with prothioconazole, the study report informs us that the field recoveries for the IOM samples are presented as prothioconazole-equivalents (i.e. the sum of prothioconazole and prothioconazole-desthio) as during the air pumping process prothioconazole is converted to prothioconazole-desthio.

Table CP 7.2.2.2/01-1: Detailed analytical results for the field recoveries spiked with prothioconazole

Fortification level of prothioconazole (µg/ sample)	Sample type	Recovery (%)		Mean recovery (%)	RSD (%)
10	Dermal dosimeter	93	101	97	8
100	Dermal dosimeter	91	100	95.5	9
1000	Dermal dosimeter	99	106	102.5	7
0.1	IOM filters	82	90	86	8
1	IOM filters	95	99	97	4

Table CP 7.2.2.2/01-2: Detailed analytical results for the field recoveries spiked with prothioconazole-desthio

Fortification level of prothioconazole- desthio (µg/ sample)	Sample type	Recovery (%)		Mean recovery (%)	RSD (%)
10	Dermal dosimeter	97	91	94	6
100	Dermal dosimeter	85	90	87.5	5
1000	Dermal dosimeter	94	92	93	2
0.1	IOM filters	81	87	84	6
1	IOM filters	94	98	96	4

Results

The actual field residue data have been corrected for field recoveries if the average field recovery for the most relevant spiking level/sampling matrix is below 95%. The correction factor is chosen based on whether the reported residue level in the actual field samples for each sample was nearer to the low, medium or high spiking level.

The amount of residues found on each mannequin's dermal dosimeters and IOM filters was used to calculate potential and actual dermal exposure as well as inhalation exposure.

- **Potential dermal exposure** (PDE) is calculated as the sum of residues on the outer clothing dosimeters (short sleeved T-shirt, shorts), the ski mask and inner clothing dosimeters (long sleeved shirt and long johns). This is intended to represent dermal exposure to a person when there is no protection from clothing.
- **Actual dermal exposure** (ADE) is calculated as the sum of residues on inner clothing dosimeters (long sleeved shirt and long johns) and ski mask. This is intended to represent dermal exposure to a person wearing shorts and t-shirt.
- All air sampling pumps showed that the average flow rate was 2 L/min ± 10%.

- **Inhalation exposure of adults** is calculated by multiplying the sum of the residues in the filter and on the cassette with 4.79 to scale up from the pump rate of 2 L/min to the standard ventilation rate for an adult of 0.23 m^3/day/kg bw = 13.8 m^3/day/ 60 kg adult = 9.58 L/min/ 60 kg adult (EFSA guidance).
- **Inhalation exposure of a child** is calculated by multiplying the sum of the residues in the filter and on the cassette by a factor of 3.715 to scale up to the standard ventilation rate for a child of 1.07 m^3/day/ kg bw = 10.7 m^3/day/ 10 kg child = 7.43 L/min/ 10 kg child (EFSA guidance).

Table CP 7.2.2.2/01-3: Prothioconazole residues on outer and inner dermal dosimeters and IOM filters from child mannequins

Distance from the zero line	2 m			5 m			8 m		
Mannequin ID	**A1**	**A2**	**A3**	**B1**	**B2**	**B3**	**C1**	**C2**	**C3**
Outer clothing (µg/sample)									
TOTAL	26.4	42.7	60	12.1	9.51	8.14	13.6	12.9	5.08
Inner clothing (µg/sample)									
TOTAL	24.3	24.1	28.3	7.37	7.25	7.53	9.62	6.98	5.02
IOM samplers (µg/sample)									
Filter	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1
Cassette	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1
TOTAL	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2
Values in red are <LOQ and have been reported at the LOQ									
Operator	**A1**	**A2**	**A3**	**B1**	**B2**	**B3**	**C1**	**C2**	**C3**
Outer clothing (mg)	0.0264	0.0427	0.0600	0.0121	0.0095	0.0081	0.0136	0.0129	0.0051
Inner clothing (mg)	0.0243	0.0241	0.0283	0.0074	0.0073	0.0075	0.0096	0.0070	0.0050
Air sampler (mg)	0.0002	0.0002	0.0002	0.0002	0.0002	0.0002	0.0002	0.0002	0.0002
PDE(mg/person)	0.0507	0.0668	0.0883	0.0195	0.0168	0.0157	0.0232	0.0199	0.0101
ADE (mg/person)	0.0243	0.0241	0.0283	0.0074	0.0073	0.0075	0.0096	0.0070	0.0050
PIE (mg/person)	0.0007	0.0007	0.0007	0.0007	0.0007	0.0007	0.0007	0.0007	0.0007

Table CP 7.2.2.2/01-4: Prothioconazole residues on outer and inner dermal dosimeters and IOM filters from adult mannequins

Distance from the zero line	2 m			5 m			8 m		
Mannequin ID	**A1**	**A2**	**A3**	**B1**	**B2**	**B3**	**C1**	**C2**	**C3**
Outer clothing (µg)									
TOTAL	101	61.4	81.1	27.8	20	24.8	21.9	12.5	10.7
Inner clothing (µg)									
TOTAL	47.8	65.7	78.2	21.1	17.8	19.5	15.8	13.1	8.35
IOM samplers (µg)									
Filter	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1
Cassette	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1
TOTAL	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2
Values in red are <LOQ and have been reported at the LOQ									
Mannequin ID	A1	A2	A3	B1	B2	B3	C1	C2	C3
Outer clothing (mg)	0.1010	0.0614	0.0811	0.0278	0.0200	0.0248	0.0219	0.0125	0.0107
Inner clothing (mg)	0.0478	0.0657	0.0782	0.0211	0.0178	0.0195	0.0158	0.0131	0.0084
Air sampler (mg)	0.0002	0.0002	0.0002	0.0002	0.0002	0.0002	0.0002	0.0002	0.0002

PDE(mg/person)	0.1488	0.1271	0.1593	0.0489	0.0378	0.0443	0.0377	0.0256	0.0191
ADE (mg/person)	0.0478	0.0657	0.0782	0.0211	0.0178	0.0195	0.0158	0.0131	0.0084
PIE (mg/person)	0.0010	0.0010	0.0010	0.0010	0.0010	0.0010	0.0010	0.0010	0.0010

Table CP 7.2.2.2/01-5: Prothioconazole-desthio values on outer and inner dermal dosimeters and IOM filters from child mannequins

Mannequin distance from the zero line	**2 m**			**5 m**			**8 m**		
Mannequin ID	**A1**	**A2**	**A3**	**B1**	**B2**	**B3**	**C1**	**C2**	**C3**
Outer clothing (µg)									
TOTAL	6.1	9.4	19.3	3.5	2.4	1.9	2.8	2.0	1.0
Inner clothing (µg)									
TOTAL	12.6	13.6	16.3	3.7	3.6	3.4	3.3	2.4	1.2
IOM samplers (µg)									
Filter	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1
Cassette	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1
TOTAL	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2
Values in red are <LOQ and have been reported at the LOQ									
	Samples corrected for 94% recovery								
Operator	**A1**	**A2**	**A3**	**B1**	**B2**	**B3**	**C1**	**C2**	**C3**
Outer clothing (mg)	0.0061	0.0094	0.0193	0.0035	0.0024	0.0019	0.0028	0.0020	0.0010
Inner clothing (mg)	0.0126	0.0136	0.0163	0.0037	0.0035	0.0034	0.0033	0.0024	0.0012
Air sampler (mg)	0.0002	0.0002	0.0002	0.0002	0.0002	0.0002	0.0002	0.0002	0.0002
PDE(mg/person)	0.0187	0.0230	0.0355	0.0073	0.0058	0.0053	0.0061	0.0044	0.0022
ADE (mg/person)	0.0126	0.0136	0.0163	0.0037	0.0035	0.0034	0.0033	0.0024	0.0012
PIE (mg/person)	0.0007	0.0007	0.0007	0.0007	0.0007	0.0007	0.0007	0.0007	0.0007

Table CP 7.2.2.2/01-6: Prothioconazole-desthio residues on outer and inner dermal dosimeters and IOM filters from adult mannequins

Mannequin distance from the zero line	**2 m**			**5 m**			**8 m**		
Mannequin ID	**A1**	**A2**	**A3**	**B1**	**B2**	**B3**	**C1**	**C2**	**C3**
Outer clothing (µg)									
TOTAL	22.2	15.4	39.6	7.4	4.5	5.6	4.7	2.5	2.1
Inner clothing (µg)									
TOTAL	31.9	31.8	71.0	14.4	9.9	11.0	7.8	7.2	3.4
IOM samplers (µg)									
Filter	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1
Cassette	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1
TOTAL	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2
Values in red are <LOQ and have been reported at the LOQ									
	Samples corrected for 94% recovery								
	Samples corrected for 87.5% recovery								
Mannequin ID	**A1**	**A2**	**A3**	**B1**	**B2**	**B3**	**C1**	**C2**	**C3**
Outer clothing (mg)	0.0222	0.0154	0.0396	0.0074	0.0045	0.0056	0.0047	0.0025	0.0021
Inner clothing (mg)	0.0319	0.0318	0.0710	0.0144	0.0099	0.0110	0.0078	0.0072	0.0034
Air sampler (mg)	0.0002	0.0002	0.0002	0.0002	0.0002	0.0002	0.0002	0.0002	0.0002
PDE(mg/person)	0.0541	0.0472	0.1105	0.0217	0.0144	0.0165	0.0125	0.0098	0.0055
ADE (mg/person)	0.0319	0.0318	0.0710	0.0144	0.0099	0.0110	0.0078	0.0072	0.0034

PIE (mg/person)	0.0010	0.0010	0.0010	0.0010	0.0010	0.0010	0.0010	0.0010	0.0010

For calculation of the summary statistics for prothioconazole and prothioconazole-desthio, only actual exposure values (i.e. exposure values for a person wearing shorts and t-shirt) for mannequins positioned 2 meters from the zero line are considered based on the EFSA guidance (EFSA Journal 2014;12(10):3874, 55 pp.,) which concludes that 2 meters represents a realistic worst-case distance. As all of the inhalation sampling media (i.e. the filter and cassette) yield exposure below the LOQ, summary statistics have only been calculated for dermal exposure and these can be found Table CP 7.2.2.2/01-6 for prothioconazole and Table CP 7.2.2.2/01-7 for prothioconazole-desthio.

Table CP 7.2.2.2/01-6: Summary statistics for dermal exposure to prothioconazole based on actual dermal exposure values for adult and child mannequins positioned 2 meters from the zero line

Statistic	Adults (mg/day)	Children (mg/day)
Mean	0.0639	0.0256
Empirical 75th percentile	0.0720	0.0263
Empirical 95th percentile	0.0770	0.0279
Maximum	0.0782	0.0283
Parametric 75th percentile	0.0792	0.0278
Parametric 95th percentile	0.1453	0.0346
Log normally distributed	Yes	Yes

Table CP 7.2.2.2/01-7: Summary statistics for prothioconazole-desthio based on actual exposure values for adult and child mannequins positioned 2 meters from the zero line

Statistic	Adults (mg/day)	Children (mg/day)
Mean	0.0449	0.0141
Empirical 75th percentile	0.0514	0.0149
Empirical 95th percentile	0.0671	0.0160
Maximum	0.0710	0.0163
Parametric 75th percentile	0.0643	0.0159
Parametric 95th percentile	0.1978	0.0220
Log normally distributed	No	Yes

Data Point:	KCP 7.2.2.2/02
Report Author:	[redacted]; [redacted]
Report Year:	2015
Report Title:	Amendment no.1 to final report of study ID: P-666-15-1700 - Dermal exposure of bystanders / residents to prothioconazole and its main metabolite prothioconazole-desthio from tractor mounted/trailed boom sprayers with Aviator XPRO EC 225 in cereals
Report No:	P666151700
Document No:	M-536654-02-1
Guideline(s) followed in study:	OECD Guidance Document for the Conduct of Studies of Occupational Exposure to Pesticides During Agricultural Application, Series on Testing and Assessment No. 9, 1997 Equipment for crop protection - Methods for field measurement of spray drift, ISO 22866:2005(E)
Deviations from current test guideline:	None
Previous evaluation:	yes, evaluated and accepted Prothioconazole RAR (2018)
GLP/Officially recognised testing facilities:	Yes, conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	Yes

In this study only dermal exposure of bystanders/residents to prothioconazole and prothioconazole-desthio is measured. No measurement of inhalation exposure has been performed. The report submitted includes an amendment from the original study report. According to the authors the original study report lacks an interpretation of the exposure results therefore result interpretation and weather monitoring data were added.

Executive summary

The purpose of the study was to determine the dermal exposure of bystanders/residents to prothioconazole and its main metabolite prothioconazole-desthio from spray drift at 2 and 5 meters downwind from the sprayed area, during application of 'Aviator Xpro EC 225' in winter wheat. 'Aviator Xpro EC 225' is formulated as an emulsifiable concentrate comprising the two active ingredients prothioconazole (150 g/L) and bixafen (75 g/L).

The study was conducted on commercial agricultural land in Germany in May 2015. Headland was selected to obtain a track as near as possible to 90° to the prevailing wind direction, representing worst case conditions to persons in the vicinity affected by drift. The crop (wheat) reached a canopy height of about 60 cm and was at growth stage BBCH 56. The crop was grown on a non-sloped area of more than 10 ha in size. An area of 100 m x 20 m was mulched in the centre of the field to allow the positioning of the mannequins in the spray drift zone. The area adjacent to this zone was sprayed with two spray swaths. Each spray swath covered an area of 100 m x 18 meters equating to a total area sprayed of 0.36 ha. The pictures provided in the study report demonstrate that the first spray swath was closest to the mannequins (i.e. for the first spray swath mannequins were either 2 or 5 meters away from the zero line). The second spray swath was completed adjacent to the initial spray swath therefore the bystanders were further away from the zero line (i.e. bystanders were approximately 20 or 23 meters away from the zero line based on a spray swath of 18 meters). The zero line was set at a distance of half of the nozzle spacing from the edge of boom; as the nozzles were spaced 0.5 meters apart, the zero line can be calculated as 0.25 meters from the edge of the boom.

Spray application was performed using 'Aviator Xpro® EC 225' with a dose rate of 1.25 L/ha (corresponding to 187.5 g prothioconazole/ha) using standard spray nozzles (Type: TeeJet XR 110-03). The application was conducted with a spray swath of 18 m width, a spray volume of 100 L/ha, a forward speed of 9 km/h and a spray pressure of 1.3 bar. The spray boom was positioned at 1.1 m (0.5 m above canopy height of approximately 0.6 m). Figure CP 7.2.2.20/2-1 presents the trial layout, while Figure CP 7.2.2.20/2-2 provides visual application details for both studies.

Figure CP 7.2.2.20/2-1: Trial layout

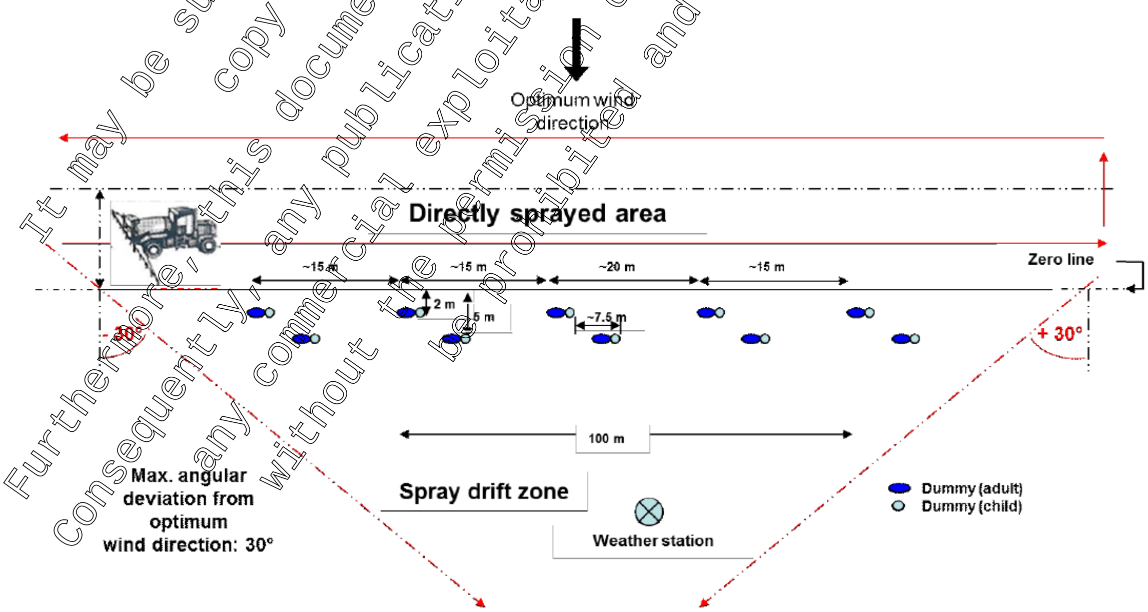
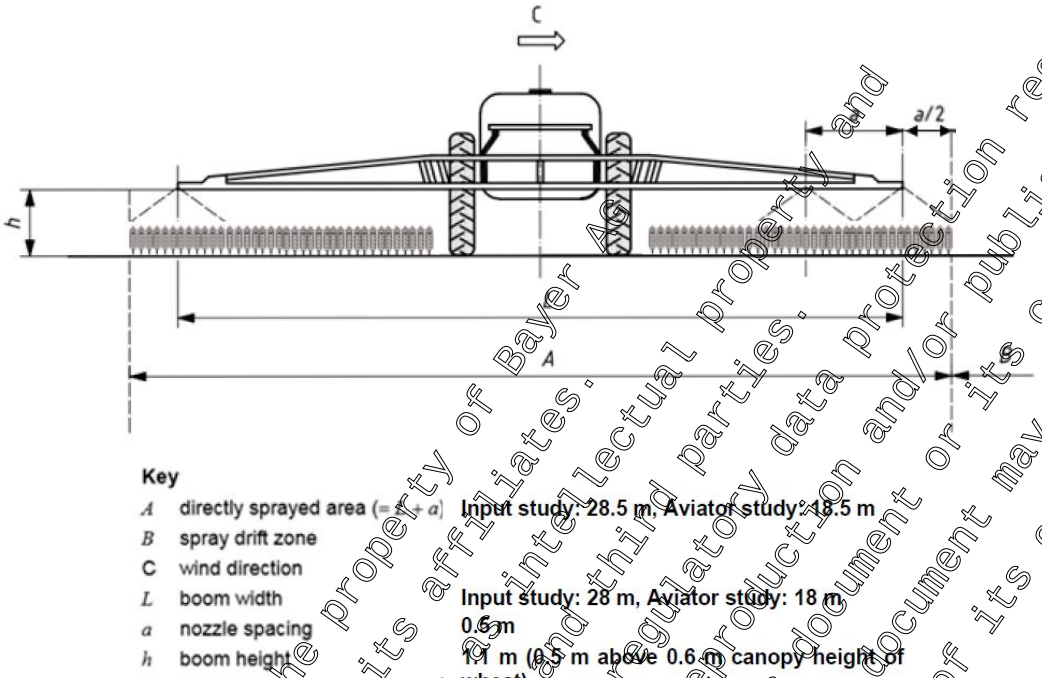


Figure CP 7.2.2.20/2-1: Application details and zero line in both studies



A comparison of the 'Aviator Xpro EC 225' study parameters with the proposed use of 'Aviator Xpro EC 225' is given in Table B.6.4.2.3- 11 which confirms that the application performed in the study was identical to the critical GAP for the representative product 'Aviator Xpro EC 225'. In addition, a comparison of the spray application in the study with the BREAM calculator is given in Table CP 7.2.2.2/02-2.

Table CP 7.2.2.2/02-1: Comparison of critical GAP for bystander/resident exposure for the proposed use of 'Aviator Xpro EC 225' and the 'Aviator Xpro EC 225' bystander/resident study

	Critical GAP for the proposed use of 'Aviator Xpro EC 225'	'Aviator Xpro EC 225' study
Product used	'Aviator Xpro EC 225'	'Aviator Xpro EC 225'
Active substance	150 g prothioconazole /L (75 g bixafen /L)	150 g prothioconazole /L (75 g bixafen /L)
Application method	Field crop boom sprayer	Field crop boom sprayer
Crop Type	Cereals	Cereals
Outdoor/protected	Outdoor	Outdoor
Maximum individual dose	1.25 L product/ha (187.5 g prothioconazole/ha and 94 g bixafen/ha)	1.25 L product/ha (187.5 g prothioconazole/ha and 94 g bixafen/ha)
Maximum number of applications	2	1
Minimum application interval (days)	14	n.a.
Maximum total dose	2.5 L product/ha (375 g prothioconazole/ha (and 188 g bixafen/ha))	n.a.

Table CP 7.2.2.2/02-2: Comparison of the spray application parameter applied in the study and the BREAM calculator

	BREAM calculator	CP 7.2.2.2/01; CP 7.2.2.2/02
Bystander type	Adult and Child	Adult and Child
Exposure route	Dermal and inhalation	Dermal

Nozzle type:	Flat Fan 03110	TeeJet XR 110-03/ flat fan
Number of nozzles:	48	36
Pressure:	3 bar	1.3 bar
Forward/Driving speed:	12.6 km/h	9 km/h
Boom height:	0.7 m	0.5 m
Crop height:	short	0.6 m
Average wind speed:	2.7 m/s	2.0-6.0 m/s (average 3.8 m/s)
Spray concentration:	1 g a.s./L spray	1.875 g a.s./ L

A. Sampling:

The test system consisted of 20 mannequins (10 adult and 10 child mannequins). The mannequins intended to represent adults were 1.86 meters high and the mannequins intended to represent children were 1.04 meters high. Five pairs of adult-child replicates were positioned 2 meters from the zero line with a distance of at least 15 meters between each pair and 5 adult-child pairs were positioned 5 meters from zero line with a distance of at least 15 meters between each pair. To avoid the mannequins positioned 2 meters from the zero line having an impact on the mannequins positioned 5 meters from the zero line, the downstream mannequin pairs were laterally displaced with a distance of 7.5 meters. The zero line was set at a distance of half the nozzle spacing from the edge of boom; as the nozzles were spaced 0.5 meters apart, the zero line can be calculated as 0.25 meters from the edge of the boom.

Wind direction, wind speed, air temperature, relative humidity, dew point and air pressure were monitored under no GLP conditions during the day of application at a frequency of 1 Hz.

Mannequins wore whole body dosimeters (all dosimeters made of 100% cotton except shorts consisting of 65% polyester / 35% cotton) but not personal air sampling devices. The dermal dosimeters consisted of long underwear [long sleeved shirts and long johns worn below short outer dosimeters (short sleeved T-shirt and shorts)] to represent persons wearing light clothing. The long sleeves of the shirts were worn over the hand therefore an additional hand dosimeter was not required. A ski mask was donned to measure total head exposure.

On completion of the spraying, mannequins were left in the field for 30 minutes to allow aerosols to settle before dosimeters were removed from the mannequins. The outer dosimeters (short sleeved T-shirt and shorts) were removed first and both samples were combined and put into pre-labelled HDPE-bottles. The inner dosimeters (long sleeved shirts, long johns) and the ski mask were then collected. These samples were also combined and put into pre-labelled HDPE-bottles. All samples were directly transported to the test facility and were placed in frozen storage at approximately -20 °C on receipt. The storage period of the frozen samples is not mentioned in the study however the study report states that the field recovery samples were collected, stored, handled and analysed in the same way as actual field samples.

B. Field recovery samples:

Field recoveries were prepared for the dermal dosimeters and were used to demonstrate the stability of prothioconazole and prothioconazole-desthio in the field samples and check the performance of the analytical method. At the beginning of the day, study personnel fortified samples of whole-body dosimeter sections with known quantities of prothioconazole and its main metabolite prothioconazole-desthio. Dosimeter samples were exposed to ambient conditions at a near location but isolated from the test plot to avoid contamination. At the end of the work day, these field spike samples were collected, stored, handled and analysed in the same way as the actual field samples. The process of fortification of dermal dosimeters is not described in the study report thus it is not clear if fortification was performed using the pesticide formulation in the spray matrix (water) as recommended by the OECD guidance document. In addition, only two replicates for each fortification level were performed. This is not in line with the OECD guidance which recommends that at least three replicates per fortification level should be performed and no explanation for the deviation from the guidance is given. Furthermore, blank control recovery samples were not carried out.

Actual field samples are corrected for field recoveries when the relevant field recovery is < 95%. The correction factor was chosen based on whether the reported residue levels in the actual field samples were nearer to the low, medium or high spiking level. For prothioconazole the fortification levels were 50, 100 and 1000 µg/sample with respective average field recoveries of 107, 112 and 102 %. These recoveries were sufficient for all levels, so no data correction was performed. For prothioconazole-desthio the fortification levels were 20, 100 and 1000 µg/sample with respective field recoveries of 96, 93 and 92%. These recovery values were sufficient (> 95%) for the fortification level of 20 µg/L only however since the amount of prothioconazole-desthio residues in all the field samples are closest to the 20 µg/sample spiking level, no correction of the data was necessary. The LOQ for prothioconazole and prothioconazole-desthio was 6 µg/sample for dermal dosimeters.

C. Method of analysis:

Residues of prothioconazole and prothioconazole-desthio in the dosimeters for dermal and inhalation exposure were determined by using the analytical method MR-13/106. MR-13/106 is an extension of 00598, which was originally evaluated for the Annex 1 inclusion, and which has been adapted to the matrices of operator and bystander exposure studies. All extensions of method 00598 were summarised in report MR-13/106 (KCA 4.1.2/42) and consequently the method is now occasionally referred to as Method MR-13/106. All areas of method 00598 which were not originally evaluated for the Annex 1 inclusion are evaluated in the Draft (Renewal) Assessment Report (DAR), Volume 3 – B.5. Thus, all methods referring to MR-13/106 can also be considered to be fully validated.

Results

Field residue data are presented in the tables below. The amount of residues found on each mannequin's dosimeter was used to calculate potential and actual dermal exposure. Potential dermal exposure was regarded as the sum of residues on the outer body dosimeters (short sleeved T-shirt, shorts), the ski mask and inner body dosimeters (long sleeved shirt and long johns) and is intended to represent dermal exposure when there is no protection from clothing. Actual dermal exposure was the sum of residues on inner dosimeters (long sleeved shirt and long johns) and ski mask and is intended to represent dermal exposure for a person wearing shorts and t-shirt. Prothioconazole field data for child and adult mannequins can be found in Table CP 7.2.2.2/02-3 and Table CP 7.2.2.2/02-4 respectively. Prothioconazole-desthio field data for child and adult mannequins can be found in Table CP 7.2.2.2/02-5 and Table CP 7.2.2.2/02-6., respectively.

Table CP 7.2.2.2/02-3: Prothioconazole residues on outer and inner dermal dosimeters on child mannequins

Mannequin distance from the zero line	**2 m**					**5 m**				
Mannequin ID	**A1**	**A2**	**A3**	**A4**	**A5**	**B1**	**B2**	**B3**	**B4**	**B5**
Outer clothing (µg)										
TOTAL	6	9.41	17	39.8	49.4	6	6	28.4	12.4	31.4
Inner clothing (µg)										
TOTAL	6	9.27	23	62.9	67.6	6	7.82	32.2	17.3	48.3
Values in red are <LOQ and have been reported at the LOQ										
Mannequin ID	A1	A2	A3	A4	A5	B1	B2	B3	B4	B5
Outer clothing (mg)	0.006	0.009	0.017	0.040	0.049	0.006	0.006	0.028	0.012	0.031
Inner clothing (mg)	0.006	0.009	0.023	0.063	0.068	0.006	0.008	0.032	0.017	0.048
PDE (mg/person)	0.012	0.019	0.040	0.103	0.117	0.012	0.014	0.061	0.030	0.080
ADE (mg/person)	**0.006**	**0.009**	**0.023**	**0.063**	**0.068**	0.006	0.008	0.032	0.017	0.048

Table CP 7.2.2.2/02-4: Prothioconazole residues on outer and inner dermal dosimeters on adult mannequins

Mannequin distance from the zero line	2 m					5 m				
Mannequin ID	**A1**	**A2**	**A3**	**A4**	**A5**	**B1**	**B2**	**B3**	**B4**	**B5**
Outer clothing (µg)										
TOTAL	6	25.4	60.2	81.7	99.5	6	9.61	22.2	42.6	50
Inner clothing (µg)										
TOTAL	6	26.1	66.4	112	120	6	14.3	46	34.8	84.3
Values in red are <LOQ and have been reported at the LOQ										
Mannequin ID	A1	A2	A3	A4	A5	B1	B2	B3	B4	B5
Outer clothing (mg)	0.006	0.025	0.060	0.082	0.100	0.006	0.010	0.022	0.043	0.050
Inner clothing (mg)	0.006	0.026	0.066	0.112	0.120	0.006	0.014	0.046	0.035	0.084
PDE(mg/person)	0.012	0.052	0.127	0.194	0.220	0.012	0.024	0.068	0.077	0.134
ADE (mg/person)	**0.006**	**0.026**	**0.066**	**0.112**	**0.120**	0.006	0.014	0.046	0.035	0.084

Table CP 7.2.2.2/02-5: Prothioconazole-desthio residues on outer and inner dermal dosimeters on child mannequins

Mannequin distance from the zero line	2 m					5 m				
Mannequin ID	**A1**	**A2**	**A3**	**A4**	**A5**	**B1**	**B2**	**B3**	**B4**	**B5**
Outer clothing (µg)										
TOTAL	6	6	6.13	8.84	10.3	6	6	9.72	6.26	9.05
Inner clothing (µg)										
TOTAL	6	6	7.2	16.8	13.6	6	6	11.2	6.79	13.6
Values in red are <LOQ and have been reported at the LOQ										
Mannequin ID	A1	A2	A3	A4	A5	B1	B2	B3	B4	B5
Outer clothing (mg)	0.006	0.006	0.006	0.009	0.010	0.006	0.006	0.010	0.006	0.009
Inner clothing (mg)	0.006	0.006	0.007	0.017	0.014	0.006	0.006	0.011	0.007	0.014
PDE(mg/person)	0.012	0.012	0.013	0.026	0.024	0.012	0.012	0.021	0.013	0.023
ADE (mg/person)	**0.006**	**0.006**	**0.007**	**0.017**	**0.014**	0.006	0.006	0.011	0.007	0.014

Table CP 7.2.2.2/02-6: Prothioconazole-desthio residues on outer and inner dermal dosimeters on adult mannequins

Mannequin distance from the zero line	2 m					5 m				
Mannequin ID	**A1**	**A2**	**A3**	**A4**	**A5**	**B1**	**B2**	**B3**	**B4**	**B5**
Outer clothing (µg)										
TOTAL	6	10.1	16	21	25.6	6	6.7	8.66	16.7	19.7
Inner clothing (µg)										
TOTAL	6	17.2	26.7	44.1	31.6	6	10.2	25.1	16.9	34.6
Values in red are <LOQ and have been reported at the LOQ										
Mannequin ID	A1	A2	A3	A4	A5	B1	B2	B3	B4	B5
Outer clothing (mg)	0.006	0.010	0.016	0.021	0.026	0.006	0.007	0.009	0.017	0.020
Inner clothing (mg)	0.006	0.017	0.027	0.044	0.032	0.006	0.010	0.025	0.017	0.035
PDE(mg/person)	0.012	0.027	0.043	0.065	0.057	0.012	0.017	0.034	0.034	0.054
ADE (mg/person)	**0.006**	**0.017**	**0.027**	**0.044**	**0.032**	0.006	0.010	0.025	0.017	0.035

The EFSA guidance (EFSA Journal 2014;12(10):3874,pg 27) informs us that the dermal exposure values for spray drift for estimating resident exposure are based on the 75th percentile exposure value from the BREAM data for mannequins positioned 2 meters from the sprayer. It also proposes that an adjustment for light clothing for residents/bystanders is appropriate. Based on this information, the

RMS has performed statistical analysis on the actual dermal exposure values for prothioconazole and prothioconazole-desthio for adult and child mannequins positioned 2 meters from the zero line and these are presented in Table CP 7.2.2.2/02-7 and Table CP 7.2.2.2/02-8, respectively.

Table CP 7.2.2.2/02-7: Summary statistics for prothioconazole based on actual dermal exposure values for adult and child mannequins positioned 2 meters from the zero line

Statistic	Adults	Children
	mg/day	mg/day
Mean	0.0661	0.0338
Empirical 75th percentile	0.1120	0.0629
Empirical 95th percentile	0.1184	0.0667
Maximum	0.1200	0.0676
Parametric 75th percentile	0.1179	0.0541
Parametric 95th percentile	0.1258	0.0279
Log normally distributed	Yes	Yes

Table CP 7.2.2.2/08: Summary statistics for prothioconazole-desthio based on actual dermal exposure values for adult and child mannequins positioned 2 meters from the zero line

Statistic	Adults	Children
	mg/day	mg/day
Mean	0.0251	0.0099
Empirical 75th percentile	0.0316	0.0136
Empirical 95th percentile	0.0416	0.0162
Maximum	0.0441	0.0168
Parametric 75th percentile	0.0388	0.0133
Parametric 95th percentile	0.1258	0.0279
Log normally distributed	Yes	Yes

RMS PL: Previous RMS UK concerns to use the submitted drift studies as a higher tier approach. During stop-the-clock, App. submitted an additional wind tunnel study that validates the studies (CP 7.2.2.2/03, M-642728-01-1). This study has been summarized below.

Data Point:	KCP 7.2.2.2/03
Report Author:	[redacted]; [redacted]; [redacted]; [redacted]
Report Year:	2018
Report Title:	Comparison of drift potential for two nozzle/pressure/forward speed combinations
Report No:	M-642728-01-1
Document No:	M-642728-01-1
Guideline(s) followed in study:	OECD guidance document for the conduct of studies of occupational exposure to pesticides during agricultural application, Series on Testing and Assessment No. 9, 1997 Equipment for crop protection - Methods for field measurement of spray drift, ISO 22866:2005(E)
Deviations from current test guideline:	None
Previous evaluation:	No, submitted, not evaluated Prothioconazole RAR (2018)
GLP/Officially recognised testing facilities:	No, not conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	Yes

Introduction and objective

The parameters in the above mentioned drift studies are slightly different than those in BREAM. A well-recognised and independent CRO (Silsoe spray application unit) conducted several wind tunnel experiments to identify a validation factor that could be applied to make a comparison between BREAM and both studies possible. The objective of this wind tunnel study was to establish the difference in drift relevant to bystander exposure, between a Teejet XR110 03 VP nozzle at 1.3 bar and 9 km/h and a conventional flat fan 110 03 nozzle (Hypro) at 3.0 bar by measurements of Droplet size distribution using laser diffraction and wind tunnel measurements of spray drift.

Materials and Methods

A. Droplet size measurements:

Measurements of the spray characteristics were made using the Malvern SprayTec laser diffraction instrument and standard protocols for spray classification. This involves a single, long-axis scan across the fan at a distance of 250 mm below the nozzle. Three replicate measurements were made for each. The spray liquid was tap water. The canister was placed on weighing scales and pressurised to dispense the liquid to the nozzle, with bespoke software monitoring the scales to determine the flow rate.

B. Wind tunnel measurements

The wind tunnel testing methodology, which was similar to the LERAP star rating protocol, is given in detail in the Appendix. The main features are:

- A moving nozzle, operated at a controlled speed across the wind tunnel
- Passive line collectors mounted at 0.1 – 0.6 m above the floor of the wind tunnel at a distance of 2.25 m downwind of the centre of the nozzle
- The nozzle mounted at 0.5 m above the lowest collector (0.6 m above the floor)
- A tracer dye was present in the spray liquid
- The quantity of spray deposited on the collecting lines was determined using spectrophotometry.

A vertical profile of drift was measured at 2.25 m downwind of the nozzle because this was considered the measurement most relevant to bystander exposure, and matched the distance used in the field trial.

Results

A. Measurements of droplet size:

Measurements of the spray characteristics of the two nozzles are given below.

Table CP 7.2.2.2/03-1: Spray characteristics measured by laser diffraction

	XR 110 03 @ 1.3 bar	FF 110 0 @ 3.0 bar
Volume median diameter (VMD)	237	168
% liquid vol. <100 µm	9	19
Estimated fan angle	133	105
Flow rate, L/min	0.763	1.201

The percentage of spray liquid contained in droplets smaller than 100 µm is significantly greater with the FF 110 03 at 3.0 bar – more than twice as much. If the absolute quantity of spray is calculated, by multiplying by the flow rate, the quantity of spray liquid emitted in droplets smaller than 100 µm is 69.4 ml/min for the XR nozzle, and 228.3 ml/min for the conventional FF nozzle.

This suggests that there is more than 3 times as much spray in fine droplets for the FF 110 03 nozzle than in the nozzle/pressure used in the field trial. However, this does not take account of the different forward speeds which were used in the field trials.

The fan angles for the two nozzles are also very different, with the XR nozzle giving a much higher fan angle than its nominal 110°. A wider fan is usually associated with higher drift because of a lower average vertical velocity for the spray droplets.

B. Measurements of spray drift

The mean values of measured drift are summarised in the following table.

Table CP 7.2.2.2/03-2 :Mean quantity of spray liquid collected on lines in the wind tunnel

	Application 1 9.0 km/h forward speed Teejet XR110 03, 1.3 bar	Application 2 12.6 km/h forward speed FF110 03, 3.0 bar
	µL spray liquid	
Height above ground (m)	Mean ±SD	Mean ±SD
0.6	0.43 ±0.15	0.38 ±0.00
0.5	1.12 ±0.21	0.46 ±0.22
0.4	3.85 ±0.51	1.82 ±0.58
0.3	13.01 ±1.67	12.33 ±1.75
0.2	23.46 ±4.86	34.71 ±3.98
0.1	26.92 ±3.80	41.58 ±3.76

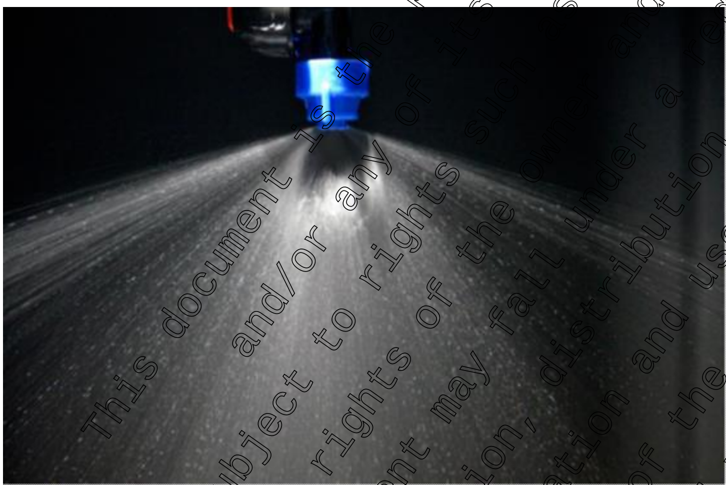
Total (µL spray liquid)	68.81	91.28
Relative drift (appli. 1/appli. 2)	1.33	
Nozzle flow rate (L/min)	0.763	1.205
Volume applied (L/ha)	101.8	114.5
Normalised drift (per 100 L/ha)	67.59	79.52
Relative normalised drift (appli. 1/appli. 2)	1.18	

Discussion

A. Spray characteristics:

The quantity of spray contained in droplets smaller than 100 µm is often taken as a surrogate measurement for spray drift, and there are many studies that show there is a high correlation between the two. However, droplet velocity is also a very important parameter and a low velocity spray will drift more than a high velocity one with the same droplet size distribution. Laser diffraction does not measure droplet velocities, but the extremely wide angle with the XR nozzle that was estimated from the measurements would be expected to have a significant impact on drift. A photograph taken of the spray fan (Figure CP 7.2.2.2/03-1) suggests that the fan angle measured this way (which is how it is defined in the ISO standard) is around 145o, even at 1.3 bar.

Figure CP 7.2.2.2/03-1: Spray fan from the XR 110 03 nozzle at 1.3 bar.



A previous study estimated the relationship between pressure and the velocity of the liquid sheet emerging from the nozzle, which influences both the initial droplet velocity and the velocity of the entrained air, both of which influence drift

The equation sheet velocity (m/s) = 13.532 x pressure (bar)$^{0.5}$ - 2.7 was found to apply to both conventional and XR nozzles (Butler Ellis and Tuck 2012), and suggests that at 1.3 bar, the sheet velocity would be around 13 m/s and at 3.0 bar, 21 m/s. This would be expected to have a very significant effect on spray drift. The only way to take all variables (droplet size, velocity, flow rate and fan angle) into account in a theoretical analysis would be to obtain the relevant data for the XR nozzle and run the Silsoe Spray Drift Model. The alternative approach is to evaluate this empirically, as is was done in this experiment, in the wind tunnel.

B. Wind tunnel measurements:

These results suggest that, had the same field experiment been conducted with the FF 110 03 nozzle at 3.0 bar at 12.6 km/h, the measurements of spray liquid deposited on the bystanders would have been, on average, approximately 1.33 higher than were actually measured. When the applied volume is taken into account (i.e. a higher applied volume results in a lower concentration of active substance for the same dose) the relative drift for the active substance is 1.18. The increased values of drift at greater heights above the ground with the XR nozzle is a reflection of its wider fan angle. The total dermal

exposure of a bystander is quantified independently of its height above the ground (e.g. there is no discrimination of whether it is deposited on the feet or on the head) and so this does not impact upon the results. The inhalation spray exposure, however, which is strongly dependent on the height above the ground, is likely to be greater with the XR nozzle in field conditions than the above results suggest because of the wide angle, and a conversion factor of less than one could be appropriate.

Conclusion

Dermal exposure to active substance can be increased by a factor of 1.18 to take account of these differences. The residue value from both studies were multiplied with this factor.

Calculation of exposure to spray drift based on study data

The EFSA guidance (EFSA Journal 2014;12(10):3874, 55 pp.,) informs us that the exposures from spray drift for residents should be calculated using the following equation:

Dermal exposure × dermal absorption percentage + inhalation exposure

where the dermal absorption percentage is the value for the in-use dilution taken from the toxicological evaluation and the dermal and inhalation exposure are based on the default 75th percentile values provided in Table 16 of the EFSA guidance.

Instead of using the default dermal exposure values in the EFSA guidance, the spray drift assessment has been refined using the highest 75th percentile and mean dermal exposure values measured from the two submitted bystander/resident studies. The summary statistics for prothioconazole and prothioconazole-desthio from the two bystander/resident exposure studies are summarised in **Table CP 7.2.2.2/03-3** and **Table CP 7.2.2.2/03-4** respectively. These have been calculated based on actual exposure values (*i.e.* exposure values which take into account protection from light clothing) from mannequins placed 2 meters from the sprayed area. For prothioconazole the highest exposure values were seen in the 'Aviator Xpro EC 225' and for prothioconazole-desthio the highest exposure values were found in the 'Input' study. Using a precautionary approach, the highest values across both studies were used in calculations.

Table CP 7.2.2.2/03-3: Summary of statistics for prothioconazole from the two bystander/resident exposure studies

Exposure scenario	Statistic	'Input' study	'Aviator Xpro EC 225' study
		mg/day**	**mg/day****
Adult	95th percentile*	0.0923 (0.0782)	0.1416 (0.1200)
	75th percentile*	0.0923 (0.0782)	0.1389 (0.1177)
	Mean	0.0754 (0.0639)	0.0800 (0.0661)
Child	95th percentile*	0.0334 (0.0283)	0.0798 (0.0676)
	75th percentile*	0.0328 (0.0278)	0.0742 (0.0629)
	Mean	0.0303 (0.0256=	0.0399 (0.0338)

* The highest of the empirical and parametric 75th /95th percentile values has been presented. In case that these values were higher than the maximum, the maximum values was considered.

** Dermal exposure to active substance can be increased by a factor of 1.18. The residue value from both studies were multiplied with this factor. Values as measure are presented in brackets. Value corrected with the proposed validation factor are highlighted with yellow shading.

Table CP 7.2.2.2/03-4: Summary of statistics for prothioconazole-desthio from the two bystander/resident exposure studies

Exposure scenario	Statistic	'Input' study	'Aviator Xpro EC 225' study
		mg/day**	**mg/day****

Adult	95th percentile*	0.0838 (0.0710)	0.0520 (0.0441)
	75th percentile*	0.0759 (0.0643)	0.0458 (0.0388)
	Mean	0.0530 (0.0449)	0.0296 (0.0251)
Child	95th percentile*	0.0192 (0.0163)	0.0198 (0.0168)
	75th percentile*	0.0188 (0.0159)	0.0163 (0.0136)
	Mean	0.0166 (0.0141)	0.0117 (0.0099)

* The highest of the empirical and parametric 75th /95th percentile values has been presented. In case that these values were higher than the maximum, the maximum values was considered.

** Dermal exposure to active substance can be increased by a factor of 1.18. The residue value from both studies were multiplied with this factor. Values as measure are presented in brackets. Value corrected with the proposed validation factor are highlighted with yellow shading.

Only the 'Input®' study measured inhalation exposure and all measurements observed were below the detection limit of the analytical method. In addition the 'Input' study only had three adult and three child mannequins placed 2 meters downwind from a passing sprayer resulting in a limited number of replicates for inhalation dosimeters. Based on this, default inhalation exposure values from the EFSA guidance have been used to estimate inhalation exposure; the default 75th percentile inhalation exposure (ml spray dilution/person) is 0.00010 for an adult and 0.00022 for a child; the default mean inhalation exposure (mL spray dilution/person) is 0.00009 for an adult and 0.00017 for a child.

With the approach detailed, acceptable bystander/resident exposure scenarios are achieved, however further data is available, which can be provided if requested (refer to Table CP 7.2.2/03-5)

Table CP 7.2.2.2/03-5: Further bystander studies available

Exposure Scenario	Analytes	Report Number	Study title
Bystander/resident vapour exposure	PTZ, PTZ-desthio, SPX	M-632396-02-1	Inhalation exposure of bystanders/residents to spiroxamine, tebuconazole and prothioconazole-desthio via vapour following tractor mounted/trailed boom sprayer application of PTZ+SPX+TBZ EC 425 in cereals
Bystander/resident direct drift	PTZ, PTZ-desthio, SPX	M-510345-01-1	Exposure of bystanders/residents to spiroxamine and prothioconazole from spray applications with Input® in cereals using drift reducing spray nozzles
Bystander/resident direct drift	PTZ, PTZ-desthio	M-536654-01-1	Dermal exposure of bystanders/residents to prothioconazole and its main metabolite prothioconazole-desthio from tractor mounted/trailed boom sprayers with Aviator XPRO EC 225 in cereals
Bystander/resident direct drift	PTZ, PTZ-desthio	M-691458-01-1	Dermal exposure of bystanders/residents to Prothioconazole and its metabolite prothioconazole-desthio from tractor mounted/trailed boom sprayers equipped with standard spray nozzles with BIX+PTZ EC 225 (75 +150) in cereals
Bystander/resident direct drift	PTZ, PTZ-desthio	M-691460-01-1	Dermal exposure of bystanders/residents to prothioconazole and its metabolite prothioconazole-desthio from tractor mounted/trailed boom sprayers equipped with drift reducing nozzles with BIX+PTZ EC 225 (75 + 150) in cereals
Bystander/resident direct drift, summary	PTZ, PTZ-desthio	M-682712-03-1	Summary document: Bystander drift studies on the dermal exposure to prothioconazole and its main metabolite, prothioconazole-desthio using standard and drift reducing nozzles
DFR	SPX	M-474542-01-1	Determination of the dislodgeable foliar residues of spiroxamine in/on wheat after spraying of JAU 6476 & KWG 4168 EC 460 in the field in Portugal

DFR	SPX	M-474550-01-1	Determination of the dislodgeable foliar residues of spiroxamine in/on wheat after spraying of JAU 6476 & KWG 4168 EC 460 in the field in Germany

CP 7.2.3 Worker exposure

Worker exposures from re-entry to treated crops were estimated using the EFSA (2015) model. This assessment provides a conservative estimate of re-entry worker exposure when inspection and irrigation activities are assessed for field (low) crops (cereals). Prothioconazole + Spiroxamine EC 460 (160+300 g/L) is used as a fungicide where there is no need to re-enter the treated area after application. Therefore, a worse-case scenario of 2 hours has been considered for crop inspection/irrigation activities.

A summary of the estimated exposure of workers to spiroxamine as a result of the critical exposure scenarios with and without the use of PPE are shown in Table CP 7.2.3-1.

Table CP 7.2.3-1 Summary of estimations of worker exposure in relation to the AOEL following crop inspection, using DFR data

Model data	Level of PPE	Total absorbed dose (mg/kg bw/d)	% AOEL	Reference
EFSA model • 0.2 kg PTZ/ha • Work rate 2 h/day[1] • DT_{50}: 30 days • DFR: 0.4656 µg/cm²/kg a.s./ha • Outdoor • 2 applications (14 day interval application)	Potential exposure[2]	0.0314	15.72	Table CP 7.2.3.2-3 (input parameter) Table CP 7.2.3.2-4 (exposure estimate)
	Work wear[3]	0.0035	1.76	
	Work wear[2] + gloves	---[4]	---[4]	
EFSA model • 0.2 kg PTZ desthio/ha • Work rate 2 h/day[1] • DT_{50}: 30 days • DFR: 0.1992 µg/cm²/kg a.s./ha • Outdoor • 2 applications (14 day interval application)	Potential exposure[2]	0.0134	134.48	Table CP 7.2.3.2-5 (input parameter) Table CP 7.2.3.2-6 (exposure estimate)
	Work wear[3]	0.0015	15.06	
	Work wear[2] + gloves	---[4]	---[4]	
EFSA model • 0.375 kg SPX/ha • Work rate 2 h/day[1] • DT_{50}: 30 days • DFR: 0.4835 µg/cm²/kg a.s./ha • Outdoor • 2 applications (14 day interval application)	Potential exposure[2]	0.0286	190.98	Table CP 7.2.3.2-7 (input parameter) Table CP 7.2.3.2-8 (exposure estimate)
	Work wear[3]	0.0032	21.39	
	Work wear[2] + gloves	---[4]	---[4]	

1 2 h/day for professional applications for inspection and irrigation
2 No work wear
3 Clothing covering arms, body, legs
4 Data not available in the EFSA model to estimate systemic exposure when PPE are worn

Conclusion

The algorithms used to estimate operator exposures are embedded in the model and use data from the 75th percentile.

According to the EFSA model calculations, when DFR generated data are used to refine the default value, it can be concluded that the risk the potential exposure for operators exposed to the active ingredients, prothioconazole (its metabolite, prothioconazole-desthio) and spiroxamine in Prothioconazole + Spiroxamine EC 460 is acceptable following application to field (low) crops. When work clothing is considered, systemic exposure is further reduced.

As a standard rule, it should be mentioned on the label that treated crops should not be re-entered before spray deposits on leaf surfaces have completely dried.

CP 7.2.3.1 Estimation of worker exposure

The exposure estimates for worker re-entry to treated crops are calculated using the EFSA (2015) model. All assumptions made in the model are explained in the EFSA guidance and are not detailed here. A summary is provided.

For a conservative Tier 1 assessment, it is assumed that no work wear is worn. However, it is considered that workers will wear clothing covering the arms, body and legs under normal circumstances and that this is a more realistic scenario.

The initial DFR (dislodgeable foliar residue) was estimated using the conservative default assumption that an application rate of 1 kg a.s./ha corresponds to an initial DFR of 3 µg/cm^2. This DFR estimate becomes even more conservative for days after application as spiroxamine is expected to dissipate and degrade on the foliage over time. No decline of residues between application and worker re-entry was considered, which represents a worst-case assumption. The maximum application rate Prothioconazole + Spiroxamine EC 460 (160+300 g/L) applied to the representative crop was used to estimate worst-case potential worker exposure after application for the particular crop for which worker exposure was being estimated. In the absence of DFR data the default DFR value has been used.

In the absence of data and based on the EFSA guidance, the following transfer coefficients (TC) were assumed:

Table CP 7.2.3.1-1 Summary of transfer coefficient values for representative crops

Crop	Transfer coefficient (cm^2/h)		
	Total potential exp.	Arms, body, legs covered	Hands, arms, body, legs covered[1]
Cereals	12500	1400	-

1 This assumes that PPE in the form of gloves are worn. For cereals however TC values to model this scenario are not available

Table CP 7.2.3.1-2 Summary of estimations of worker exposure in relation to the AOEL following crop inspection

Model data	Level of PPE	Total absorbed dose (mg/kg bw/d)	% AOEL	Reference
EFSA model • 0.2 kg PTZ/ha • Work rate 2 h/day[1] • DT$_{50}$: 30 days • DFR: 3 µg/cm^2/kg a.s./ha • Outdoor • 2 applications (14 day interval application)	Potential exposure[2]	0.2025	101.26	Table CP 7.2.1.1-4 (input parameter) Table CP 7.2.3.1-4 (exposure estimate)
	Work wear[3]	0.0227	11.34	
	Work wear[2] + gloves	---[4]	---[4]	
EFSA model • 0.2 kg PTZ desthio/ha • Work rate 2 h/day[1] • DT$_{50}$: 30 days • DFR: 3 µg/cm^2/kg a.s./ha	Potential exposure[2]	0.2025	**2025.27**	Table CP 7.2.1.1-7 (input parameter) Table CP 7.2.3.1-5 (exposure estimate)
	Work wear[3]	0.0227	**226.83**	

• Outdoor • 2 applications (14 day interval application)	Work wear[2] + gloves	---[4]	---[4]	
EFSA model • 0.375 kg SPX/ha • Work rate 2 h/day[1] • DT_{50}: 30 days • DFR: 3 µg/cm²/kg a.s./ha • Outdoor • 2 applications (14 day interval application)	Potential exposure[2]	0.1777	**1185.00**	Table CP 7.2.1.1-10 (input parameter) Table CP 7.2.3.1-6 (exposure estimate)
	Work wear[3]	0.199	**132.72**	
	Work wear[2] + gloves	---[4]	---[4]	
EFSA model • 0.2 kg PTZ desthio/ha[5] • Work rate 2 h/day[1] • DT_{50}: 30 days • DFR: 3 µg/cm²/kg a.s./ha • Outdoor 2 applications (14 day interval application)	Potential exposure[2]	0.1013	506.32	Table CP 7.2.1.1-4 (input parameter) Table CP 7.2.3.1-7 (exposure estimate)
	Work wear[3]	0.0113	56.71	
	Work wear[2] + gloves	---[4]	---[4]	

Absorbed dose values presented in **bold** exceed the assigned AOEL.

1 2 h/day for professional applications for inspection and irrigation
2 No work wear
3 Clothing covering arms, body, legs
4 Data not available in the EFSA model to estimate systemic exposure when PPE are worn
5 Consideration of 50% conversion of prothioconazole to prothioconazole desthio

Table CP 7.2.3.1-4 Worker exposure (long term exposure) results for field application of Prothioconazole + Spiroxamine EC 460 (160+300 g/L) to cereals (0.2 kg PTZ/ha), no refinement

Worker - Inspection,	Potential exposure (mg/kg bw/day)	0.2025	% of RVNAS	101.26%
	Working clothing (mg/kg bw/day)	0.0227	% of RVNAS	11.34%
	Working clothing and gloves (mg/kg bw/day)		% of RVNAS	

Table CP 7.2.3.1-5 Worker exposure (long term exposure) results for field application of Prothioconazole + Spiroxamine EC 460 (160+300 g/L) to cereals (0.20 kg PTZ desthio/ha), no refinement

Worker - Inspection,	Potential exposure (mg/kg bw/day)	0.2025	% of RVNAS	2025.27%
	Working clothing (mg/kg bw/day)	0.0227	% of RVNAS	226.83%
	Working clothing and gloves (mg/kg bw/day)		% of RVNAS	

Table CP 7.2.3.1-6 Worker exposure (long term exposure) results for field application of Prothioconazole + Spiroxamine EC 460 (160+300 g/L) to cereals (0.375 kg SPX/ha), no refinement

Worker - Inspection,	Potential exposure (mg/kg bw/day)	0.1013	% of RVNAS	1012.64%
	Working clothing (mg/kg bw/day)	0.0113	% of RVNAS	113.42%
	Working clothing and gloves (mg/kg bw/day)		% of RVNAS	

Taking the approach presented in Table CP 7.2.3.1-3, refinement to the worst case scenario is required for prothioconazole-desthio for worker re-entry activities. Refinement of exposure to prothioconazole-

desthio is estimated assuming 100% conversion for inhalation exposure and 50% conversion for dermal exposure, however the former is not relevant for outdoor worker activity, with focus on dermal exposure only. In addition, no correction with respect to the molar ratio is made.

Table CP 7.2.3.1-7 Worker exposure (long term exposure) results for field application of Prothioconazole + Spiroxamine EC 460 (160+300 g/L) to cereals (0.20 kg PTZ-desthio/ha) with refinement considerations

Worker - Inspection,	Potential exposure (mg/kg bw/day)	0.1013	% of RVNAS	506.32%
	Working clothing (mg/kg bw/day)	0.0113	% of RVNAS	56.71%
	Working clothing and gloves (mg/kg bw/day)		% of RVNAS	

Dermal exposure from the 'potential exposure' and 'working clothing' adjust in EFSA model to take by 50% conversion of prothioconazole to prothioconazole-desthio, with EFSA model input parameters for prothioconazole remaining unchanged.

Conclusion

The algorithms used to estimate worker exposures are embedded in the model and use data from the 75[th] percentile.

According to the EFSA model calculations it can be concluded that the risk for workers exposed to the active ingredient, prothioconazole (and its metabolite, prothioconazole-desthio) in Prothioconazole + Spiroxamine EC 460 (160+300 g/L) is acceptable with workwear clothing following application to field (low) crops. Taking this approach for the active ingredient spiroxamine within the formulation, worker exposure exceeds the AOEL. In order to obtain acceptable exposure, DFR generated data has been used to refine the risk assessment.

CP 7.2.3.2 Measurement of worker exposure

Collectively, estimated worker exposure to prothioconazole as well as to the metabolite, prothioconazole-desthio can be estimated using data from the worker re-entry study conducted with Prothioconazole + Tebuconazole 250 g/L EC applied to barley.

With the approach adopted during the previous renewal process, and detailed under CP 7.2.1.22, the data have been used in a generic manner as opposed to address the special issue of prothioconazole-desthio. The study data lend itself to be used in exposure assessments for formulations containing additional active substances apart from prothioconazole. To serve for this purpose the data are expressed as "prothioconazole equivalents", *i.e.* the results of prothioconazole-desthio are converted to "prothioconazole results" (by multiplication with 1.103, derived from the molar ratio) and added to the results of prothioconazole, giving "prothioconazole-equivalents".

Table CP 7.2.3.2-1: Collective overview of dislodgeable foliar residues (DFR) of prothioconazole – equivalents normalized/kg/ha applied

Days after application	Prothioconazole (µg/cm² of foliage/kg a.s. applied)	Prothioconazole-desthio (µg/cm² of foliage/kg a.s. applied)	Prothioconazole-equivalents (µg/cm² of foliage/kg a.s. applied)
0	<0.005	<0.005	<0.005
1	0.4608	0.1384	0.3299
3	<0.005	<0.005	<0.005
7	<0.005	<0.005	<0.005
10	<0.005	<0.005	<0.005
14/0	0.1112	0.1808	0.4835
0	0.4656	0.1992	<0.005
1	0.3528	0.0976	0.3898
3	0.1992	0.0451	0.1648
7	0.0800	<0.005	0.1378
10	0.07048	<0.005	<0.005

With the normalised DFR data, this can be input into the EFSA model.

The results show that there is a rapid dissipation for prothioconazole, prothioconazole-desthio and tebuconazole. No increase or accumulation of residues on the leave surface was observed with the second application. From the data, a Tier I, worst case assessment has been taken using the highest normalized DFR value obtained for each compound.

Table CP 7.2.3.2-2: Summary of estimations of worker exposure in relation to the AOEL following crop inspection, using DFR data

Model data	Level of PPE	Total absorbed dose (mg/kg bw/d)	% AOEL	Reference
EFSA model • 0.2 kg PTZ/ha • Work rate 2 h/day[1] • DT_{50}: 30 days • DFR: 0.4656 µg/cm²/kg a.s./ha • Outdoor • 2 applications (14 day interval application)	Potential exposure[2]	0.0314	15.72	Table CP 7.2.3.2-3 (input parameter) Table CP 7.2.3.2-4 (exposure estimate)
	Work wear[3]	0.0035	1.76	
	Work wear[2] + gloves	---[4]	---[4]	
EFSA model • 0.2 kg PTZ desthio/ha • Work rate 2 h/day[1] • DT_{50}: 30 days • DFR: 0.1992 µg/cm²/kg a.s./ha • Outdoor • 2 applications (14 day interval application)	Potential exposure[2]	0.0134	134.48	Table CP 7.2.3.2-5 (input parameter) Table CP 7.2.3.2-6 (exposure estimate)
	Work wear[3]	0.0015	15.06	
	Work wear[2] + gloves	---[4]	---[4]	
EFSA model • 0.375 kg SPX/ha • Work rate 2 h/day[1] • DT_{50}: 30 days • DFR: 0.4835 µg/cm²/kg a.s./ha • Outdoor • 2 applications (14 day interval application)	Potential exposure[2]	0.0286	190.98	Table CP 7.2.3.2-7 (input parameter) Table CP 7.2.3.2-8 (exposure estimate)
	Work wear[3]	0.0032	21.39	
	Work wear[2] + gloves	---[4]	---[4]	

1 2 h/day for professional applications for inspection and irrigation
2 No work wear
3 Clothing covering arms, body, legs
4 Data not available in the EFSA model to estimate systemic exposure when PPE are worn

Conclusion

The algorithms used to estimate operator exposures are embedded in the model and use data from the 75th percentile.

According to the EFSA model calculations, when DFR generated data are used to refine the default value, it can be concluded that the risk the potential exposure for operators exposed to the active ingredients, prothioconazole (its metabolite, prothioconazole-desthio) and spiroxamine in Prothioconazole + Spiroxamine EC 460 is acceptable following application to field (low) crops. When work clothing is considered, systemic exposure is further reduced.

As a standard rule, it should be mentioned on the label that treated crops should not be re-entered before spray deposits on leaf surfaces have completely dried.

Table CP 7.2.3.2-3: Input parameters for the EFSA model for the active substance prothioconazole when applied to cereals (field), tier II assessment for worker exposure, with DFR refinement [also used to refine entry to crops for residents and bystanders]

Substance name	Prothioconazole (PTZ)	
Product name	PTZ + SPX EC 460 (160+300 g/L)	
Reference value non acutely toxic active substance (RVNAS)	0.2	mg/kg bw/day
Reference value acutely toxic active substance (RVAAS)		mg/kg bw/day
Crop type	Cereals	
Substance properties		
Formulation type	Soluble concentrates, emulsifiable concentrate, etc.	
Miniumum volume water for application (liquids)	100	L/ha
Maximum application rate of active substance	0.2	kg a.s./ha
50% Dissipation Time DT_{50}	30	days
Initial Dislodgeable Foliar Residue	0.4656	µg/cm² of foliage/kg a.s. applied/ha
Dermal absorption of product	25.00%	
Dermal absorption of in-use dilution	47.00%	
Oral absorption of active substance	100.00%	
Inhalation absorption of active substance	100.00%	
Vapour pressure of active substance	low volatile substances having a vapour pressure of <5*10-3Pa	
Scenario		
Indoor or Outdoor application	Outdoor	
Application method	Downward spraying	
Application equipment	Vehicle-mounted-Drift Reduction	
Buffer strip	2-3	m
Number of applications	2	
Interval between multiple applications	14	days
Season (upward spraying orchards only)	not relevant	

Table CP 7.2.3.2-4 Worker exposure (long term exposure) results for field application of Prothioconazole + Spiroxamine EC460 (160+300 g/L) to cereals (0.2 kg PTZ/ha), with DFR data

Worker - Inspection	Potential exposure (mg/kg bw/day)	0.0314	% of RVNAS	15.72%
	Working clothing (mg/kg bw/day)	0.0035	% of RVNAS	1.76%
	Working clothing and gloves (mg/kg bw/day)		% of RVNAS	

Table CP 7.2.3.2-5: Input parameters for the EFSA model for the metabolite prothioconazole-desthio when applied to cereals (field), tier II assessment for worker exposure, with DFR refinement [also used to refine entry to crops for residents and bystanders]

Substance name	Prothioconazole-desthio (PTZ-desthio)	
Product name	PTZ + SPX EC 460 (160+300 g/L)	
Reference value non acutely toxic active substance (RVNAS)	0.01	mg/kg bw/day
Reference value acutely toxic active substance (RVAAS)		mg/kg bw/day
Crop type	Cereals	
Substance properties		
Formulation type	Soluble concentrates, emulsifiable concentrate, etc.	
Miniumum volume water for application (liquids)	100	L/ha
Maximum application rate of active substance	0.2	kg a.s./ha
50% Dissipation Time DT_{50}	30	days
Initial Dislodgeable Foliar Residue	0.1992	$\mu g/cm^2$ of foliage/kg a.s. applied/ha
Dermal absorption of product	25.00%	
Dermal absorption of in-use dilution	47.00%	
Oral absorption of active substance	100.00%	
Inhalation absorption of active substance	100.00%	
Vapour pressure of active substance	low volatile substances having a vapour pressure of <5*10-3Pa	
Scenario		
Indoor or Outdoor application	Outdoor	
Application method	Downward spraying	
Application equipment	Vehicle-mounted-Drift Reduction	
Buffer strip	2-3	m
Number of applications	2	
Interval between multiple applications	14	days
Season (upward spraying orchards only)	not relevant	

Table CP 7.2.3.2-6: Worker exposure (long term exposure) results for field application of Prothioconazole + Spiroxamine EC460 (160+300 g/L) to cereals (0.20 kg PTZ desthio/ha), with DFR data

Worker Inspection,	Potential exposure (mg/kg bw/day)	0.0134	% of RVNAS	134.48%
	Working clothing (mg/kg bw/day)	0.0015	% of RVNAS	15.06%
	Working clothing and gloves (mg/kg bw/day)		% of RVNAS	

Table CP 7.2.3.2-7: Input parameters for the EFSA model for the active substance spiroxamine when applied to cereals (field), tier II assessment for worker exposure, with DFR refinement [also used to refine entry to crops for residents and bystanders]

Substance name	Spiroxamine (SPX)	
Product name	PTZ + SPX EC 460 (160+300 g/L)	
Reference value non acutely toxic active substance (RVNAS)	0.015	mg/kg bw/day
Reference value acutely toxic active substance (RVAAS)	0.061	mg/kg bw/day
Crop type	Cereals	
Substance properties		
Formulation type	Soluble concentrates, emulsifiable concentrate, etc.	
Miniumum volume water for application (liquids)	100	L/ha
Maximum application rate of active substance	0.375	kg a.s./ha
50% Dissipation Time DT_{50}	30	days
Initial Dislodgeable Foliar Residue	0.4835	µg/cm² of foliage/kg a.s. applied/ha
Dermal absorption of product	0.87%	
Dermal absorption of in-use dilution	22.00%	
Oral absorption of active substance	61.00%	
Inhalation absorption of active substance	100.00%	
Vapour pressure of active substance	low volatile substances having a vapour pressure of <5*10-3Pa	
Scenario		
Indoor or Outdoor application	Outdoor	
Application method	Downward spraying	
Application equipment	Vehicle-mounted-Drift Reduction	
Buffer strip	2-3	m
Number of applications	2	
Interval between multiple applications	14	days
Season (upward spraying orchards only)	not relevant	

Table CP 7.2.3.2-8: Worker exposure (long term exposure) results for field application of Prothioconazole + Spiroxamine EC460 (160+300 g/L) to cereals (0.375 kg SPX/ha), with DFR data

Worker - Inspection	Potential exposure (mg/kg bw/day)	0.0286	% of RVNAS	190.98%
	Working clothing (mg/kg bw/day)	0.0032	% of RVNAS	21.39%
	Working clothing and gloves (mg/kg bw/day)		% of RVNAS	

Data Point:	KCP 7.2.3.2/01
Report Author:	[redacted]; [redacted]
Report Year:	2020
Report Title:	Determination of the dislodgeable foliar residues (DFR) of prothioconazole and tebuconazole in/on barley after spray application of JAU 6476 & HWG 1608 EC 250 in Italy
Report No:	E19DF004
Document No:	M-690952-01-1
Guideline(s) followed in study:	US EPA OPPTS 875.2100 Foliar Dislodgeable Residue Dissipation
Deviations from current test guideline:	None
Previous evaluation:	yes, evaluated and accepted Prothioconazole RAR (2018)
GLP/Officially recognised testing facilities:	Yes, conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	Yes

Executive Summary

The magnitude of the dislodgeable foliar residues (DFR) of the substances prothioconazole, tebuconazole and prothioconazole-desthio was determined in/on washings from barley leaf punches after two spray applications with Prothioconazole + Tebuconazole 250 g/L EC (JAU 6476 & HWG 1608 EC 250), a emulsifiable concentrate formulation containing 125 g/L prothioconazole and 125 g/L tebuconazole.

The study included one supervised residue trial conducted in the field in Italy (Southern European Residue Zone(, during the 2019 season.

The dislodging of the leaf samples were performed no later than 4 h post collection

Under the conditions of this study, there is a rapid dissipation for prothioconazole, prothioconazole-desthio and tebuconazole. No increase or accumulation of residues on the leave surface was observed with the second application. The enantiomer ratios for prothioconazole, prothioconazole-desthio and tebuconazole remained unchanged racemic.

Analytical results (DFR; µg/cm^2) of treated samples are summarised below. No residues of prothioconazole, prothioconazole-desthio and tebuconazole above the LOQ were found in the control samples. The mean of the laboratory recoveries for prothioconazole, prothioconazole-desthio and tebuconazole were in the acceptable range of 70-110% and a relative standard deviation of <20%.

The mean of the field recovery samples for prothioconazole, prothioconazole-desthio and tebuconazole were also in the acceptable range of 70-110% and a relative standard deviation of <20%.

The results for the dislodgeable foliar residues for prothioconazole, prothioconazole-desthio and tebuconazole in the field samples were not corrected for laboratory or field spike recoveries.

The results showed that there is a rapid dissipation for prothioconazole, prothioconazole-desthio and tebuconazole. No increase or accumulation of residues on the leave surface was observed with the second application.

The enantiomer ratios for prothioconazole, prothioconazole-desthio and tebuconazole remained unchanged racemic.

Under the conditions of this study, there is a rapid dissipation for prothioconazole, prothioconazole-desthio and tebuconazole. No increase or accumulation of residues on the leave surface was observed with the second application. The enantiomer ratios for prothioconazole, prothioconazole-desthio and tebuconazole remained unchanged racemic.

Materials and methods

A. Materials:

1. Test Material:	Prothioconazole + Tebuconazole 250 g/L EC (alternative name: JAU 6476 & HWG 1608 EC 250)	
Purity:	126.3 g prothioconazole/L 124.7 g tebuconazole/L	
CAS No.:	178928-70-6 (prothioconazole) 107534-96-3 (tebuconazole)	
2. Field site:		
Location:	Italy	
Plot size:	240 m^2, divided into 3 sub plots for sampling of 80 m^2/plot	
Soil type:	Sandy clay loom	
Crop:	Barley (tektoo)	
Crop height:	0.5 m	
Date of sowing:	8 Nov 2018	
Start/end of flowering:	23 April 2019 / 30 April 2019	
	Application 1	**Application 2**
Date of application	9 April 2019	23 April 2019
Growth stage:	39 BBCH	61 BBCH
Date of harvesting:	15 June 2019 to 15 July 2019	
3. Equipment details:		
Equipment	Spray applicator, with Albuz Flat Fan Drift Guard nozzles	
Nozzle size:	CVI 11003	
No. of nozzles:	8	
Nozzle spacing (cm):	50	
Pressure:	3.0 bar (at pump)	
4. Application details:	JAU 6476 & HWG 1608 EC 250	
Product application rate (AR):	1 L product/ha	
AR of a.s.:	0.125 kg prothioconazole/ha 0.125 kg tebuconazole/ha	
Spray volume:	300 L/ha	300 L/ha
No. of applications:	1	2
Application interval:	-	14 days
5. Environmental conditions:		
Temperature at application:	12˚C	17˚C
Humidity:	54%	65%
Wind speed:	1.0 m/s	2.0 m/s
Wind direction:	West	South
Rain fall:	2.5 mm within 24 h after application	0 mm within 24 h after application
Rain fall post application:	20 h	-

B. Field sampling, transport and storage:

Planned sampling schedule details are summarized below. The washing samples were frozen as soon as possible, but not later than 12 hours after sampling until dispatch to the Laboratory for Sampling. The

storage period of deep-frozen samples used for the analysis of tebuconazole, prothioconazole and metabolites was between 291 and 315 days

Table CP 7.2.3.2/01-1: Determination of the dislodgeable foliar residues (DFR) of prothioconazole and tebuconazole on barley: sampling schedule for both test sites

Sampling event		Days after 1st application	Days after 2nd application
1/2	Prior 1st application (Control + Field Spikes)	Before application	
3	Post 1st application	0	
4	Post 1st application	1	
5	Post 1st application	3	
6	Post 1st application	7	
7	Post 1st application	10	
8	Prior 2nd application	14	Before application
9	Prior 2nd application	-	0
10	Prior 2nd application	-	1
11	Prior 2nd application	-	3
12	Prior 2nd application	-	7
13	Prior 2nd application	-	10
No. of sampling events		13	
No. of samples		12 *x* 3 = 36 + 3 additional control samples prior to 1st application for field spikes = 39	

Methods:

A. Collecting of leaf punches:

Leaf punches were collected directly into polypropylene jars using a leaf punch sampler. Each sample consisted of 80 disks cut with a leaf puncher with 1.262 cm diameter and a disk area of 1.25 cm^2. The leaf punches represented a total double-sided leaf surface area of 200 cm^2. A sample was collected from each of the three subplots to provide three replicate samplings at each sampling interval. Leaf punches were taken by chance over the inner parts of the subplots from the potential worker contact zone. Control leaf punch samples were collected prior to the first application. Treated samples collected on the day of application were taken after the spray had dried. After each sample was collected, the sampling jar was capped and transported to the field site laboratory for dislodging. Leaf punch samplers were cleaned after each sampling interval.

B. Dislodgeable foliar residue sample collection:

The dislodging of the leaf samples were performed no later than 4 h post collection. The samples were dislodged by placing leaf sampling in a surfactant (0.01% Aerosol OT solution (*i.e.* docusate sodium salt)) and placed on a shaker operating at approximately 200 cycles/minute for a period of *ca.* 10 minutes. The solution was decanted from the leaf material into an appropriate container and the dislodging procedure was repeated for each sample. The second rinse was again decanted and added to the first. Cysteine hydrochloride solution was added to stabilize the active compound prothioconazole. Immediately after dislodging the leaves, the jar was capped, labelled and placed into the deep freezer within 12 hours after sampling. The dislodged leaves were discarded.

C. Field recovery samples:

Field fortification samples were used to demonstrate the stability of the samples during storage period of the study and the ability of the analytical laboratory to recover an analyte fortified into a sample at the field test site. The solutions from dislodged control samples were fortified with tebuconazole, prothioconazole and prothioconazole-desthio at the LOQ and at a level of 10 to 200 times of the LOQ. Field spikes were performed by the field technician prior to the 1st application. The field recovery samples were treated in the same manner as the field residue samples until analysis.

LOQ was set to 0.005 µg/cm² (corresponding to 5 µg/L). Spiking levels were: 0.005 µg/cm² (corresponds to 5 µg/L), 0.05 µg/cm² (corresponds to 50 µg/L) and 1 µg/cm² (corresponds to 1000 µg/L). For each level (unspiked control, 5 µg/L, 50 µg/L and 1000 µg/L) three replicates were performed.

D. Analytical method:

In the context of the spiroxamine renewal dossier submission, the analytical method for prothioconazole / prothioconazole-desthio and tebuconazole are not discussed.

Results

A. Dislodgeable foliar residue data:

Analytical results (DFR; µg/cm^2) of treated samples are summarised below. No residues of prothioconazole, prothioconazole-desthio and tebuconazole above the LOQ were found in the control samples. The mean of the laboratory recoveries for prothioconazole, prothioconazole-desthio and tebuconazole were in the acceptable range of 70-110% and a relative standard deviation of <20%.

The mean of the field recovery samples for prothioconazole, prothioconazole-desthio and tebuconazole were also in the acceptable range of 70-110% and a relative standard deviation of <20%.

The results for the dislodgeable foliar residues for prothioconazole, prothioconazole-desthio and tebuconazole in the field samples were not corrected for laboratory or field spike recoveries.

The results showed that there is a rapid dissipation for prothioconazole, prothioconazole-desthio and tebuconazole. No increase or accumulation of residues on the leave surface was observed with the second application.

Table CP 7.2.3.2/01-1: Determination of the dislodgeable foliar residues (DFR) of prothioconazole and tebuconazole on barley: analytical results or treated sample washings from leaf punches

Days after application	Prothioconazole (µg/cm^2/0.125 kg PTZ applied)	Prothioconazole-desthio (µg/cm^2/0.125 kg PTZ-desthio applied)	Tebuconazole (µg/cm^2/0.125 kg TBZ applied))
0	<0.005	<0.005	<0.005
1	0.0576	0.0173	0.0217
3	0.0138	<0.005	<0.005
7	0.0129	<0.005	<0.005
10	0.00967	<0.005	<0.005
14/0	0.0139**	0.0226**	0.0343**
0	0.00473	<0.005	<0.005
1	0.0582	0.0249	0.0216
3	0.0441	0.0122	0.00762
7	0.0249	0.00564	0.0105
10	0.0160	<0.005	<0.005
LOQ	0.00881	<0.005	<0.005

a. sample mix up is likely but could not be identified in the chain of sampling to analysis. The results seem to be the results of DAT 1

b. reported as measured because the results for sub plots T2 and T3 are > LOQ and a clear signal were detected.

B. Enantiomer ratio:

The enantiomer ratios for prothioconazole, prothioconazole-desthio and tebuconazole remained unchanged racemic.

Table CP 7.2.3.2/01-2 Determination of the dislodgeable foliar residues (DFR) of prothioconazole and tebuconazole on barley: enantiomer ratio

Days after application	Prothioconazole	Prothioconazole-desthio	Tebuconazole
0 / 0 / 0	0.972 / 0.983 / 1.05	0.967 / 0.961	0.998 / 0.986 / 1.00

1 / 1/ 1	---[a] / ---[a] / ---[a] /	---[a] / ---[a] / ---[a] /	0.998 / 0.992 / 1.01
3 / 3 / 3	---[a] / ---[a] / ---[a] /	---[a] / ---[a] / ---[a] /	1.02 / 0.986 / 0.988
7 / 7 / 7	---[a] / ---[a] / ---[a] /	---[a] / ---[a] / ---[a] /	0.988 / 1.01 /1.01
10 / 10 /10	0.978 / 1.06 / 0.964	0.999 / 0.996 / 0.986	0.999 / 0.983 / 1.00
14/-0 / 14/-0 / 14/-0	---[a] / ---[a] / ---[a] /	---[a] / ---[a] / ---[a] /	0.960 / 0.960 / 1.04
0 / 0 / 0	0.955 / 0.944 / 0.948	0.948 / 0.970 / 0.976	1.01 / 1.02 / 1.01
1 / 1/ 1	1.15 / 0.972 / 0.999	0.951 / 0.965 / 0.985	0.996 / 0.984 / 0.983
3 / 3 / 3	0.992 / 0.923 / 1.11	0.931 / 0.936 / 0.978	0.978 / 0.995 / 0.999
7 / 7 / 7	---[a] / ---[a] / ---[a] /	---[a] / ---[a] / ---[a] /	0.984 / 0.994 / 0.981
10 / 10 /10	---[a] / ---[a] / ---[a] /	---[a] / ---[a] / ---[a] /	0.988 / 1.02 / 0.988

a. sample mix up is likely but could not be identified in the chain of sampling to analysis. The results seem to be the results of DAT 1
b. reported as measured because the results for sub-plots T2 and T3 are > LOQ and a clear signal were detected.

C. Deficiencies:

None

Assessment and conclusions by applicant:

Assessment: Study meets the current guidance and the requirements in 284/2013.

Conclusion: Under the conditions of this study, there is a rapid dissipation for prothioconazole, prothioconazole-desthio and tebuconazole. No increase or accumulation of residues on the leave surface was observed with the second application. The enantiomer ratios for prothioconazole, prothioconazole-desthio and tebuconazole remained unchanged racemic.

CP 7.3 Dermal absorption

It is acknowledged that an *in vivo* dermal absorption study conducted in Rhesus monkeys on Prothioconazole SC 480, and previously evaluated and deemed at the time accepted, is available. However, when assessed against the current EFSA dermal absorption guidance (2017) for the permitted variation for active substances in similar formulations, the permitted variation exceeds the allowed limit, and therefore is not considered suitable to use this study to evaluate the dermal absorption of prothioconazole.

Two new *in vitro* dermal absorption studies have been conducted examining prothioconazole-desthio, the metabolite of prothioconazole, (spray dilution only) and spiroxamine (concentrate and spray dilution) in the Prothioconazole + Spiroxamine EC 460.

Dermal absorption data were not generated on the formulation concentrate; therefore, a 25% default value has been applied to the concentrate for prothioconazole. To account for the degradation of prothioconazole to its metabolite, the active substance prothioconazole was replaced by prothioconazole-desthio and the lowest spray dilution (0.26 g/L) was investigated.

The *in vitro* human dermal absorption studies had an 8 hour exposure and results were interpreted in accordance with the current EFSA dermal absorption guidance. Estimated dermal absorption values of 0.87% (spray dilution equivalent to 0.9375 g/L) and 22% (concentrate) for spiroxamine and 47% (0.26 g/L) for prothioconazole-desthio were determined.

Table CP 7.3-01 Dermal absorption values for the risk assessment

Endpoint	Dermal absorption values	Reference

Dermal penetration	Prothioconazole Concentrate (default value): 25%	EFSA (2017)
	Spray dilution (0.26 g/L [1:615 dilution]): 47% (prothioconazole-desthio used as a substitute)	CP 7.3/01 M-758748-01-1
	Prothioconazole-desthio Spray dilution (0.26 g/L [1:615 dilution]): 47%	
	Spiroxamine Concentrate (300 g/L): 0.87% Spray dilution: (0.9375g/L [1:320 dilution]): 22%	CP 7.3/02 M-762905-01-1

In vitro dermal absorption in human skin

Data Point:	KCP 7.3/01
Report Author:	[redacted]; [redacted]
Report Year:	2020
Report Title:	Prothioconazole-desthio - The in vitro percutaneous absorption of radiolabelled prothioconazole-desthio in a single in-use dilution of the PTZ-SPX EC 460 formulation through human split-thickness skin
Report No:	786454
Document No:	M-758748-01-1
Guideline(s) followed in study:	OECD Guideline for Testing of Chemicals, Guideline 428: Skin Absorption: In Vitro Method (2004). OECD Environmental Health and Safety Publications Series on Testing and Assessment No. 28. Guidance Document for the Conduct of Skin Absorption Studies (2004). Guidance on Dermal Absorption (EFSA Journal, 2017; 15(6): 4873).
Deviations from current test guideline:	None
Previous evaluation:	No, not previously submitted
GLP/Officially recognised testing facilities:	Yes, conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	Yes

Executive Summary

The dermal absorption of prothioconazole-desthio from an emulsifiable concentrate (EC) formulation was studied using human skin *in vitro*. One concentration of in-use spray dilution was tested, 0.26 g/L.

The dose was applied at 10 µL/cm^2 to dermatomed split-thickness skin and left unoccluded for an experimental period of 24 h, with an interim wash at 8 h post-application and a termination wash at 24 h

The skin samples from four different donors were mounted into static diffusion cells and the diffusion cell placed in water bath maintain a temperature of 32 ±1°C. The absorption process was followed by taking samples of the receptor fluid, phosphate buffered saline containing polyoxyethylene 20 oleyl ether (PEG, *ca* 6%, w/v), sodium azide (*ca* 0.01%, w/v), streptomycin (*ca* 0.1 mg/mL) and penicillin (*ca* 100 units/mL), pH 7.47 ± 0.02, at recorded intervals throughout the experimental period.

The distribution of prothioconazole-desthio within the test system and a 24 h absorption profile was determined using liquid scintillation counting. Before conducting the main study, stability and solubility assessments were carried out. The barrier integrity was also assessed *via* electrical resistance measurement of the skin samples.

The mass balance for [^{14}C]-prothioconazole-desthio in this dilution was 98.4%. Therefore, the data absorption from all cells was not normalised to 100%, as the mass balance was consistently >95%.

The study demonstrated that the amount of prothioconazole-desthio absorbed through split-thickness over 24 h from 0.26 g/L was 35.87 ±15.35%, as measured in the exposed skin, receptor fluid and receptor wash (not normalised). Using the current EFSA Guidance on Dermal Absorption 2017, 15(6): 4873, the estimate to be used for risk assessment is 47% for 0.26 g/L.

Table CP 7.3/01-1: Prothioconazole + Spiroxamine EC 460 (160+300 g/L): summary of the mean dermal absorption results for prothioconazole-desthio[1]

Test Preparation:	**Test preparation 1**	
Target concentration (g/L)	0.26	
Actual dose (g/L)	0.50	
Number of replicates	12	
	Recovery [%]	
	Mean	S.D.
Dislodgeable dose		
Skin washing after 8 h	55.38	13.80
Skin washing after 24 h	5.48	2.85
Donor chamber wash	0.37	0.24
Dose associated to skin		
Tape strips: strips 1 + 2	0.23	0.20
Tape strips: strips 3 - 20	0.89	0.78
Unexposed skin	0.18	0.06
Absorbed dose		
Exposed skin	3.21	2.23
Receptor fluid	31.49	15.63
Receptor chamber wash	1.17	0.40
Total recovery[1]	98.40	2.90
Absorption essentially complete at end of study (>75% absorption within half the study duration) [%Absorption at $t_{0.5}$]	Yes [76.71%]	
Absorption estimate normalised[2]	No	
If no: Absorption estimates = absorbed dose + tape strips 3-20)[3]	Not applicable	
If yes: Absorption estimates = absorbed dose	35.87	15.35
Relevant absorption estimate[4]	46.92	
Absorption estimates used for risk assessment[5]	**47**	

[1] Values may not calculate exactly from the report due to rounding of figures

[2] According to the EFSA Guidance on Dermal Absorption, cells with insufficient recovery (< 95%) can be corrected by normalisation of absorption estimate to 100% recovery.

[3] In accordance with the EFSA Guidance on Dermal Absorption (EFSA Journal 2017;15(6):4873) the radioactivity in the second tape-strip pool (3rd to nth tape strip) is considered potentially absorbable if less than 75% of the absorption occurred in the first half of the study.

[4] Dermal absorption values corrected for variability (mean + 0.72×SD (n=10)), based on Table 1 from the EFSA Guidance on dermal absorption, 2017).

[5] Relevant absorption estimate was rounded to the required number of significant figures.

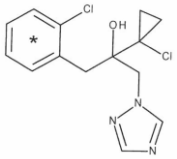
Materials and methods

A. Materials:

1. Test Material (non-radiolabelled): Prothioconazole-desthio (alternative name: PTZ-desthio)

CAS number Not assigned

Description	White crystals
Lot/Batch No.:	AE 1194888-PU-03
Purity:	98.1%
Stability of test compound:	Confirmed stable for the duration of the study (Expiry date: 17 November 2020)
2. Test Material (radiolabelled):	[cyclohex[phenyl-UL-^{14}C]-prothioconazole-desthio



* Denotes the position of ^{14}C labelled atoms

Lot/Batch No.:	KML 9576
Specific activity:	5.40 MBq/mg
Radiochemical purity:	>98%
3. Blank formulation	PTZ+SPX EC 460
Lot/Batch No.:	2020-002900*030
Description	Yellow liquid
Stability of test compound:	Confirmed stable for the duration of the study (Expiry date: 30 September 2020)
4. Test skin:	
Species:	Human
Sex:	3 ♀, 1 ♂
Age:	35 - 67 yrs
Site:	Abdomen

5. Preparation of dosing solutions

Test preparation 1: [^{14}C]-prothioconazole-desthio (145.9 µCi/mg; 0.26 mg) was transferred to a glass vial and solvent was removed. Blank premix (79.93 mg) was added to the vial and mixed by vortex. Ultrapure water (418 µL) was added to a vial in aliquots already containing blank formulation and vortexed after each addition. Six aliquots (6.4 µL) were taken, mixed with methanol: scintillant and analysed by liquid scintillation counting (LSC). The concentration of PTZ-desthio was found to be too high (195.16% of target), so ultrapure water (417 µL) was added in aliquots, mixing by vortex between each aliquot. The test preparation was mixed continuously by magnetic stirring as six more aliquots were taken and analysed as described previously. The radioactivity was quantified by LSC. The concentration of [^{14}C]-prothioconazole-desthio by radioactivity was 0.26 g/L.

B. Study Design and Methods:

1. In life dates: 22 June 2020 to 7 July 2020 (experimental dates)

2. Skin preparation: Samples of full-thickness human skin (abdomen) were obtained from three female and one male donor aged 35 to 67 years old. The samples arrived frozen and were stored in a freezer set to maintain a temperature of -20°C until used in the study.

Prior to use, the samples were removed from the freezer and allowed to reach ambient temperature prior to use. 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 thickness of the membranes was measured using a micrometer.

An electrical barrier integrity assessment was undertaken prior to treatment. Phosphate buffered saline was added to the donor chamber and the skin samples were allowed to equilibrate for *ca* 30 min. The electrical resistance was then measured using a set at low voltage alternating current, 1000 Hz with a maximum voltage of 300 mV root-mean-squared in the parallel equivalent circuit mode. Any skin sample exhibiting a resistance < 7.7 kΩ was excluded from subsequent absorption measurements. The phosphate buffered saline was removed from the skin surface and then the skin was rinsed with water and dried with tissue paper.

3. Solubility of prothioconazole-desthio in the receptor fluid:

The receptor fluid chosen for use in this study was phosphate buffered saline (PBS) containing polyoxyethylene 20 oleyl ether (6%, w/v), sodium azide (0.01%, w/v), streptomycin (0.1 mg/mL) and penicillin (100 units/mL). The pH was 7.45 – 7.49.

The solubility of spiroxamine in receptor fluid was determined to ensure that it would not reach a concentration, which would limit its diffusion. Prothioconazle-desthio was predicted to have a water solubility of 5000 mg/L measured at pH 7 (20ºC). Theoretically, if 70% of spiroxamine was absorbed, this would result in a test item concentration in the receptor fluid of 2.33 mg/L.

4. Treatment:

Split-thickness membranes (*ca* 1.5 *x* 1.5 cm) were cut and positioned into static diffusion cells. These cells were positioned in a manifold heated *via* a circulating water bath to maintain a skin surface temperature of 32 ±1 °C. The surface area of exposed skin within the cells was 0.64 cm^2, with a receptor chamber volume of 5 mL (nominally).

A single dose of 6.4 µL (10 µL/cm^2) of the test preparation was applied evenly over the surface of 12 split-thickness human skin membranes using a positive displacement pipette. The donor chambers of the cells were left non-occluded. Seven representative aliquots of each of the test preparations were dispensed into vials at the time of dosing (also referred to as mock doses), mixed with scintillation cocktail and analyzed by LSC.

5. Sampling:

Absorption of [^{14}C]-prothioconazole-desthio from the test preparation was assessed by collecting fractions of the receptor fluid at the following time intervals: 1, 2, 4, 8 and 12 h post dose.

The exposure period was terminated at 8 h post dose. Commercial hand wash soap (50 µL) was applied to the skin and the soap gently rubbed onto the skin with a cotton swab. The skin was then rinsed with approximately 5 mL of a 2% (v/v) commercial soap solution. The soap solution was applied in aliquots and each aliquot was aspirated with a pipette. The skin was dried with a cotton swab. This process was repeated once.

The soap solution (skin wash) and cotton swabs samples were mixed with scintillation cocktail and analysed using LSC.

At 24 hours post dose, *i.e.* after 16 hours monitoring period, each diffusion cell was dismantled and the skin removed. The skin was placed on a piece of tissue paper to dry the underside of the skin. The tissue was added to the receptor wash pot. Donor chambers were extracted using a solvent for *ca* 30 min before sonication (10 min). Following the removal of the apparatus, the sample was split into 5 vials. The *stratum corneum* was removed with a maximum of 20 successive tape strips. The skin sample was rotated 90° after each tape strip. Rotation was stopped if the epidermis/dermis junction became fragile or if epidermis was removed. Each tape strip was placed into an individual vial containing methanol: scintillation fluid and then analysed by liquid scintillation counting. The skin under the cell flange (unexposed skin) was cut away from the exposed skin. The exposed and unexposed skin samples were placed into separate vials containing Solvable®. The skin samples were placed into a water bath set to *ca* 60°C to aid solubilisation. Stannous chloride solution (0.2 g/mL in ethanol; 500 µL) and scintillation fluid were added to each skin sample. Samples were analysed by liquid scintillation counting.

6. Radioassay:

All samples prepared in scintillation fluid were subjected to LSC, together with representative blank samples. If necessary, samples were dissolved and/or diluted in an appropriate solvent prior to LSC analysis.

All radioactivity measurements were performed by LSC using a Packard 2100-TR scintillation counter. Where scintillation fluid was added to the samples, this was 10 mL. Where methanol:scintillation fluid was added, this was 12 mL. A limit of reliable measurement of 30 d.p.m. above background has been instituted in these laboratories.

7. Data interpretation:

Calculation of applied dose: Before, during and after dose application, mock doses were taken at an equal dose to calculate back the actual dose applied to the skin membranes.

Calculation of dermal absorption parameters: Dislodgeable dose (skin wash 8 & 24 h + tissue swab 8 & 24 h + pipette tip 8 & 24 h + donor wash), unabsorbed dose (total dislodgeable dose + *stratum corneum* + unexposed skin), absorbed dose (cumulative receptor fluid + receptor chamber wash) and dermal delivery (total absorbed dose + exposed skin) are reported as defined in OECD guidance document No. 428. Potentially absorbable dose (complete/incomplete absorption) are reported as defined in EFSA 2017 Guidance on Dermal Absorption.

Samples with a mass balance outside 90% - 110% were reviewed on a case by case basis and appropriate action justified. Where the mass balance is below 90% and the loss can be explained, the samples may be accepted.

Results

A. Dermal absorption:

1. Solubility of the test item in receptor fluid:

The solubility of prothioconazole-desthio in the receptor fluid indicated that 101.19% of the maximum applied dose could dissolve in the receptor fluid. Therefore, the test item solubility in the receptor fluid was not rate limiting.

2. Skin integrity test:

The integrity of the reported skin samples was within the acceptability criteria (absorption <7.7 kΩ). All data is presented in full in the report.

3. Test preparation 1 (nominally 0.26 g/L):

Ten samples of human split-thickness skin membranes obtained from 4 different donors were dosed topically with [^{14}C]-prothioconazole-desthio in test preparation 1 (0.26 g/L). Overall, the absorption profiles looked similar for all samples, with the absorption of [^{14}C]-prothioconazole-desthio increasing to 24 h post dose. The mass balance for all individual samples was within 100 ±10% (98.4%), with the exception of Cell 1, which had a mass balance of 94.51% of the applied dose. However, this cell was not excluded, as the absorbed dose for Cell 1 was similar to the other cells from the same donor (1274). The absorption values from donor 1247 were higher in comparison to the other donors used. Therefore, it can be assumed that the higher absorption is attributable to the donor, and that the missing material for Cell 1 is attributable to the unabsorbed dose (e.g. dislodged dose). The mean mass balance was >95% of the applied dose. Therefore it was not considered necessary to normalise to 100%, as the mass balance was consistently >95%.

The following results are provided as mean values (n = 12, not normalised):

Following the wash at 8 h, 55.38% of the applied dose of [^{14}C]-prothioconazole-desthio was washed off. At 24 h post dose, a further 5.48% was removed during the wash. A proportion of the dose applied was recovered from the donor chamber (0.37%), exposed skin (3.21%) and receptor chamber wash (1.17%). The mean total recovery was 98.40% of the applied dose

C. Deficiencies:

None.

Conclusions

Assessment and conclusion by applicant:

Assessment: This study is deemed acceptable and meets the requirements in 284/2013.

Conclusion: The study demonstrated that the amount of prothioconazole-desthio absorbed through split-thickness over 24 h from 0.26 g/L was 35.87 ±15.35%, as measured in the exposed skin, receptor fluid and receptor wash (not normalised, total recoveries >95%). Using the current EFSA Guidance on Dermal Absorption 2017, 15(6): 4873, the estimate to be used for risk assessment is 47% for 0.26 g/L.

Data Point:	KCP 7.3/02
Report Author:	[redacted]; [redacted]
Report Year:	2021
Report Title:	Spiroxamine and prothioconazole EC 460: The In Vitro percutaneous absorption of radiolabelled spiroxamine in a concentrate formulation and a single in-use dilution through human split-thickness skin
Report No:	786271
Document No:	M-762905-01-1
Guideline(s) followed in study:	OECD Guideline for Testing of Chemicals, Guideline 428: Skin Absorption: *In Vitro* Method (2004); OECD Environmental Health and Safety Publications, Series on Testing and Assessment no. 28, Guidance document for the conduct of skin absorption studies (March 2004); European Commission Guidance Document on Dermal Absorption – Sanco/222/2000/Rev. 7 (19 March 2004); Guidance on Dermal Absorption (EFSA Journal, 2017, 15(6): 4873)
Deviations from current test guideline:	None
Previous evaluation:	No, not previously submitted
GLP/Officially recognised testing facilities:	Yes, conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	Yes

Executive Summary

The dermal absorption of spiroxamine from an emulsifiable concentrate (EC) formulation was studied using human skin *in vitro*. Two concentrations were tested: a concentrate formulation of 300 g/L (test preparation 1) and an in-use spray dilution of 0.9375 g/L (test preparation 2).

The dose was applied at 10 µL/cm^2 to dermatomed/split-thickness skin and left unoccluded for an experimental period of 24 h, with an interim wash at 8 h post-application and a termination wash at 24 h.

The skin samples from four different donors were mounted into static diffusion cells and the diffusion cell placed in water bath maintain a temperature of 32 ±1°C. The absorption process was followed by taking samples of the receptor fluid, phosphate buffered saline containing polyoxyethylene 20 oleyl ether (PEG, *ca* 6%, w/v), sodium azide (*ca* 0.01%, w/v), streptomycin (*ca* 0.1 mg/mL) and penicillin (*ca* 100 units/mL), pH 7.4 ± 0.1, at recorded intervals throughout the experimental period.

The distribution of spiroxamine within the test system and a 24 h absorption profile was determined using liquid scintillation counting. Before conducting the main study, stability and solubility

assessments were carried out. The barrier integrity was also assessed *via* electrical resistance measurement of the skin samples.

The mass balance for [^{14}C]-Spiroxamine in the formulation concentrate and spray dilution were 99.82% and 94.04%, respectively. The absorption data for the spray dilution from all cells was normalised to 100%, as the mass balance from nine of twelve cells was <95%.

The study demonstrated that the amount of spiroxamine absorbed through human split-thickness skin over 24 h for the formulation concentrate (300 g/L) and spray dilution (0.9375 g/L) was 0.56 ±0.30% and 16.57 ±4.23%, respectively, as measured in the exposed skin, receptor fluid and receptor wash (normalised). Using the current EFSA Guidance on Dermal Absorption 2017, 15(6): 4873, the estimate to be used for risk assessment is 0.87% and 22% for the formulation concentrate and spray dilution, respectively.

Table CP 7.3/02-1:
Spiroxamine and Prothioconazole 460 g/L EC: summary of the mean dermal absorption results[4]

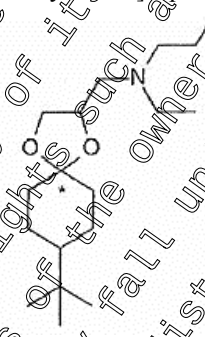
Test Preparation:	**Test preparation 1**		**Test preparation 2**	
Target concentration (g/L)	300		0.9375	
Actual dose (g/L)	232		0.9730	
Number of replicates	12		12	
	Recovery [%]			
	Mean	S.D	Mean	S.D
Dislodgeable dose				
Skin washing after 8 h	97.96	7.54	63.77	4.49
Skin washing after 24 h	0.93	0.53	10.82	2.47
Donor chamber wash	0.17	0.15	0.71	0.53
Dose associated to skin				
Tape strips: strips 1- 2	0.07	0.06	0.67	0.50
Tape strips: strips 3 - 20	0.11	0.06	1.45	1.07
Unexposed skin	0.01	0.01	0.05	0.03
Absorbed dose				
Exposed skin	0.18	0.16	2.97	1.91
Receptor fluid	0.35	0.16	12.58	3.37
Receptor chamber wash	0.03	0.01	1.02	0.49
Total recovery[1]	99.82	7.52	94.04	3.01
Absorption essentially complete at end of study (>75% absorption within half the study duration) [%Absorption at $t_{0.5}$]	No [40.0%]		No [59.8%]	
Absorption estimate normalised[2]	No		Yes	
If no: Absorption estimates = absorbed dose + tape strips 3-20)	0.67	0.30	Not applicable	
If yes: Absorption estimates = absorbed dose	Not applicable		19.13	3.88
Relevant absorption estimate	0.87		21.61	
Absorption estimates used for risk assessment[5]	**0.87**		**22**	

1 Values may not calculate exactly from the report due to rounding of figures
2 According to the EFSA Guidance on Dermal Absorption, cells with insufficient recovery (< 95%) can be corrected by normalisation of absorption estimate to 100% recovery.
3 In accordance with the EFSA Guidance on Dermal Absorption (EFSA Journal 2017;15(6):4873) the radioactivity in the second tape-strip pool (3rd to nth tape strip) is considered potentially absorbable if less than 75% of the absorption occurred in the first half of the study.
4 Dermal absorption values corrected for variability (mean + 0.64×SD (n=12)), based on Table 1 from the EFSA Guidance on dermal absorption, 2017).
5 Relevant absorption estimate was rounded to the required number of significant figures

Materials and methods

A. Materials:

1. Test Material (non-radiolabelled):	Spiroxamine
CAS number	Not stated
Description	Yellow liquid
Lot/Batch No.:	EDTH011499
Purity:	97.00%
Stability of test compound:	Confirmed stable for the duration of the study (Expiry date: 04 June 2021)
2. Test Material (radiolabelled):	[cyclohexyl-4-^{14}C]-Spiroxamine



* Denotes the position of ^{14}C labelled atoms

Lot/Batch No.:	10489IMC035-4
Specific activity:	115.2 µCi/mg
Radiochemical purity:	99.80%
3. Blank formulation	Blank Formulation of SPX+PTZ EC460
Lot/Batch No.:	2020-003239
Storage conditions	Ambient
Nominal specific gravity / density	Not applicable
4. Test skin:	
Species:	Human
Sex:	♀
Age:	38 - 64 yrs
Site:	Abdomen
5. Preparation of dosing solutions	*Test preparation 1*: The specific activity of the radiochemical had to be lowered prior to formulating test preparation 1. The solvent from 300 µL of [^{14}C]-Spiroxamine was dried off using nitrogen, technical spiroxamine (511.71 mg) was added and vortexed mixed. Analysis by liquid scintillation counting of this stock solution confirmed that [^{14}C]-Spiroxamine was homogeneously distributed in the solution with a CV of 3.51% and the specific activity was determined to be 0.509

µCi/mg. [14C]-Spiroxamine stock solution (305.64 mg) was transferred to volumetric flask, blank formulation was added to the calibration line and the contents mixed magnetic stirring plate. The radioactivity was quantified by liquid scintillation counting. The concentration of [14C]-Spiroxamine by radioactivity was 332 g/L.

Test preparation 2: [14C]-Spiroxamine (245 µL (1.8767 mg), specific activity: 115.2 µCi/mg). The solvent was removed with nitrogen gas. Blanks formulation (4.7 µL) was added to the preparation and vortex mixed. Ultrapure water (1500 µL) was added in aliquots, vortex mixing occurred between aliquots. The concentration of spiroxamine in the test preparation was too high. Therefore, the sample was further diluted with ultrapure water (400 µL) to achieve the target concentration. The radioactivity was quantified by liquid scintillation counting. The concentration of [14C]-Spiroxamine by radioactivity was 0.9730 g/L.

B. Study Design and Methods:

1. In life dates: 3 June 2020 to 23 June 2020 (experimental dates)

2. Skin preparation: Samples of full-thickness human skin (abdomen) were obtained from four female donors aged 38 to 64 years old. The samples arrived frozen and were stored in a freezer set to maintain a temperature of -20°C until used in the study. Prior to use, the samples were removed from the freezer and allowed to reach ambient temperature prior to use. 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 thickness of the membranes was measured using a micrometer.

An electrical barrier integrity assessment was undertaken prior to treatment. Phosphate buffered saline was added to the donor chamber and the skin samples were allowed to equilibrate for *ca* 30 min. The electrical resistance was then measured using a set at low voltage alternating current, 1000 Hz with a maximum voltage of 300 mV root-mean-squared in the parallel equivalent circuit mode. Any skin sample exhibiting a resistance < 7.7 kΩ was excluded from subsequent absorption measurements. The phosphate buffered saline was removed from the skin surface and then the skin was rinsed with water and dried with tissue paper.

3. Solubility of Spiroxamine in the receptor fluid: The receptor fluid chosen for use in this study was phosphate buffered saline (PBS) containing polyoxyethylene 20 oleyl ether (6%, w/v), sodium azide (0.01%, w/v), streptomycin (0.1 mg/mL) and penicillin (100 units/mL). The pH was 7.42 – 7.45.

The solubility of spiroxamine in receptor fluid was determined to ensure that it would not reach a concentration, which would limit its diffusion. Spiroxamine was predicted to have a water solubility of 340-470 mg/L measured at pH 7 (20°C). Theoretically, if 25% of spiroxamine was absorbed, this would result in a test item concentration in the receptor fluid of 96 mg/L.

4. Treatment: Split-thickness membranes (*ca* 1.5 *x* 1.5 cm) were cut and positioned into static diffusion cells. These cells were positioned in a manifold heated *via* a circulating water bath to maintain a skin surface temperature of 32 ±1 °C. The surface area of exposed skin within the cells was 0.64 cm^2, with a receptor chamber volume of 5 mL (nominally).

A single dose of 6.4 µL (10 µL/cm^2) of the test preparation was applied evenly over the surface of 12 split-thickness human skin membranes using a positive displacement pipette. The donor chambers of the cells were left non-occluded. Seven representative aliquots of each of the test preparations were dispensed into vials at the time of dosing (also referred to as mock doses), mixed with scintillation cocktail and analyzed by LSC. The same process was undertaken for both preparations.

5. Sampling: Absorption of [14C]-Spiroxamine from the test preparation was assessed by collecting fractions of the receptor fluid at the following time intervals: 1, 2, 4, 8, 12 and 24 h post dose.

The exposure period was terminated at 8 h post dose. Commercial hand wash soap (50 µL) was applied to the skin and the soap gently rubbed onto the skin with a tissue swab. The skin was then rinsed with approximately 5 mL of a 2% (v/v) commercial soap solution. The soap solution was applied in aliquots and each aliquot was aspirated three times with a pipette. The skin was dried with a tissue swab. This process was repeated and skin dried with an additional tissue swab.

The soap solution (skin wash) and tissue swabs samples were mixed with scintillation cocktail and analysed using LSC.

At 24 hours post dose, *i.e.* after 16 hours monitoring period, each diffusion cell was dismantled and the skin removed. The skin was placed on a piece of tissue paper to dry the underside of the skin. The tissue was added to the receptor wash pot. Donor chambers were extracted using a solvent for *ca* 30 min before sonication (10 min). Following the removal of the apparatus, the sample was split and mixed with scintillation fluid. The *stratum corneum* was removed with a maximum of 20 successive tape strips. The skin sample was rotated 90° after each tape strip. Rotation was stopped if the epidermis/dermis junction became fragile or if epidermis was removed. Each tape strip was placed into an individual vial containing methanol: scintillation fluid and then analysed by liquid scintillation counting. The skin under the cell flange (unexposed skin) was cut away from the exposed skin. The exposed and unexposed skin samples were placed into separate vials containing Solvable®. The skin samples were placed into a water bath set to *ca* 60°C to aid solubilisation. Stannous chloride solution (0.2 g/mL in ethanol; 500 µL) and scintillation fluid were added to each skin sample. Samples were analysed by liquid scintillation counting.

6. Radioassay: All samples prepared in scintillation fluid were subjected to LSC, together with representative blank samples. If necessary, samples were dissolved and/or diluted in an appropriate solvent prior to LSC analysis.

All radioactivity measurements were performed by LSC using a Packard 2100-TR scintillation counter. Where scintillation fluid was added to the samples, this was 10 mL. Where methanol:scintillation fluid was added, this was 12 mL. A limit of reliable measurement of 30 d.p.m. above background has been instituted in these laboratories.

7. Data interpretation: *Calculation of applied dose:* Before, during and after dose application, mock doses were taken at an equal dose to calculate back the actual dose applied to the skin membranes.

Calculation of dermal absorption parameters: Dislodgeable dose (skin wash 8 & 24 h + tissue swab 8 & 24 h + pipette tip 8 & 24 h + donor wash), unabsorbed dose (total dislodgeable dose + stratum corneum + unexposed skin), absorbed dose (cumulative receptor fluid + receptor chamber wash) and dermal delivery (total absorbed dose + exposed skin) are reported as defined in OECD guidance document No. 428. Potentially absorbable dose (complete/incomplete absorption) are reported as defined in EFSA 2017 Guidance on Dermal Absorption.

Samples with a mass balance outside 90% - 110% were reviewed on a case by case basis and appropriate action justified. Where the mass balance is below 90% and the loss can be explained, the samples may be accepted.

Results

A. Dermal absorption:

1. Solubility of the test item in receptor fluid: The solubility of spiroxamine in the receptor fluid indicated that 54% of the maximum applied dose could dissolve in the receptor fluid. Therefore, the test item solubility in the receptor fluid was not rate limiting.

2. Skin integrity test: The integrity of the reported skin samples was within the acceptability criteria (absorption <7.7 kΩ). All data is presented in full in the report.

3. Test preparation 1 (nominally 300 g/L): Twelve samples of human split-thickness skin membranes obtained from 4 different donors were dosed topically with [^{14}C]-Spiroxamine in test preparation

1 (300 g/L). Overall, the absorption profiles looked similar for all samples, with the absorption of [^{14}C]-Spiroxamine increasing to 24 h post dose. The mass balance for all individual samples was within 100 ±10%, with the exception of Cell 24, which had a mass balance of 83.82% of the applied dose. However, this cell was not excluded, as the missing material can be associated with the 8 h dislodgeable dose, and absorbed values are consistent with its donor pair (Cell 23).

The following results are provided as mean values (n = 12, not normalised): Following the wash at 8 h, 97.96% of the applied dose of [^{14}C]-Spiroxamine was washed off. At 24 h post dose, a further 0.93% was removed during the wash. A proportion of the dose applied was recovered from the donor chamber (0.17%), exposed skin (0.18%) and receptor chamber wash (0.03%). The mean total recovery was 99.82% of the applied dose.

3. Test preparation 2 (nominally 0.9375 g/L):

Twelve samples of human split-thickness skin membranes obtained from 4 different donors were dosed topically with [^{14}C]-Spiroxamine in test preparation 2 (0.9375 g/L). Overall, the absorption profiles looked similar for all samples, with the absorption of [^{14}C]-Spiroxamine increasing to 24 h post dose. The mass balance for all individual samples was within 100 ±10%. However, the mean mass balance was <95% of the applied dose. Therefore, all cells were normalised to 100%.

The following results are provided as mean values (n = 12, not normalised): Following the wash at 8 h, 63.77% of the applied dose of [^{14}C]-Spiroxamine was washed off. At 24 h post dose, a further 10.82% was removed during the wash. A proportion of the dose applied was recovered from the donor chamber (0.71%), exposed skin (2.97%) and receptor chamber wash (1.02%). The mean total recovery was 94.04% of the applied dose.

C. Deficiencies:

None.

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

Assessment: This study is deemed acceptable and meets the requirements in 284/2013.

Conclusion: The study demonstrated that the amount of spiroxamine absorbed through human split-thickness skin over 24 h for the formulation concentrate (300 g/L) and spray dilution (0.9375 g/L) was 0.56 ±0.30% and 16.57 ±4.23%, respectively, as measured in the exposed skin, receptor fluid and receptor wash (normalised). Using the current EFSA Guidance on Dermal Absorption 2017, 15(6): 4873, the estimate to be used for risk assessment is 0.87% and 22% for the formulation concentrate and spray dilution, respectively.

CP 7.4 Available toxicological data relating to co-formulants

CONFIDENTIAL information – data provided separately (Document JCP).