Document Title

Summary of the toxicological studies for Spiroxamine EC 500 (500 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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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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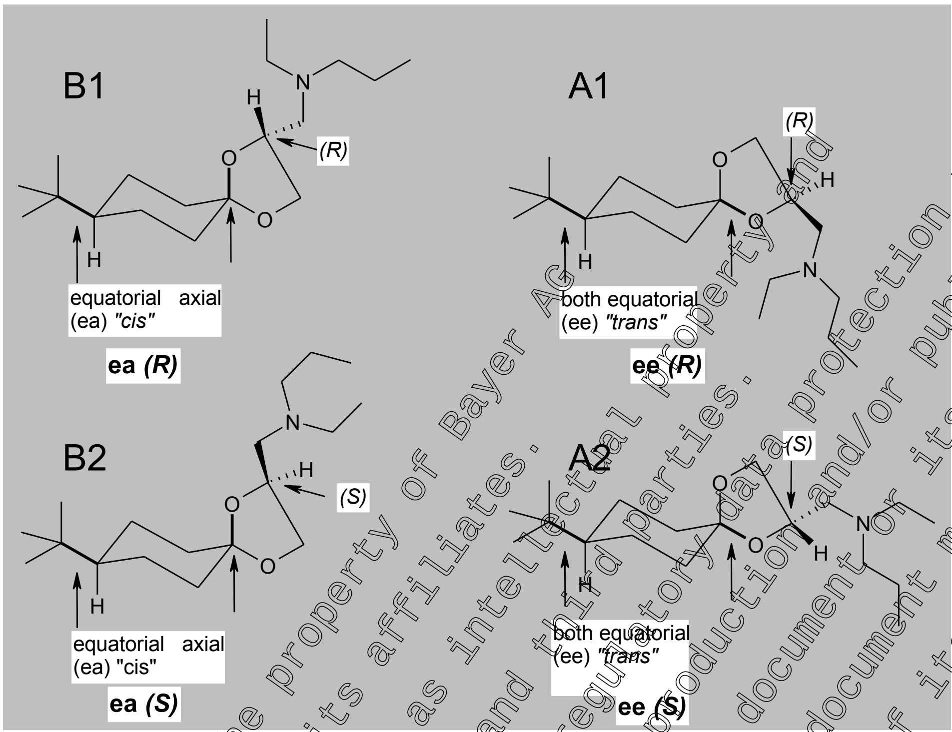
CP 7 TOXICOLOGICAL STUDIES ON THE PLANT PROTECTION PRODUCT

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 approval and subsequent 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 previous Annex I inclusion and 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 Spiroxamine EC 500 (500 g/L), abbreviation Spiroxamine EC 500, is an emulsifiable concentrate formulation containing 500 g/L of spiroxamine. This formulation is registered throughout Europe under trade names such as BAYAM, HOGGAR, IMPULSE EC 500, PROSPER, PROSPER 500 EC. Spiroxamine EC 500 was already a representative formulation of Bayer AG for the Annex I inclusion and 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

All studies for this endpoint were presented and evaluated during the EU process for the Annex I inclusion of spiroxamine under Council Directive 91/414/EEC.

Two acute oral toxicity studies confirmed Spiroxamine EC 500 (500 g/L) to be of low to moderate toxicity with LD_{50} values of 1000 mg/kg bw and >500 mg/kg bw for male and female rats, respectively. The dermal toxicity confirmed spiroxamine to be of low toxicity, with an LD_{50} >2000 mg/kg bw. A four hour nose-only acute inhalation toxicity study confirmed Spiroxamine EC 500 (500 g/L) to be of low-moderate toxicity, with an LC_{50} value of 2.323 mg/L (equivalent to a systemic exposure of 418.1 mg/kg bw).

Spiroxamine EC 500 (500 g/L) was found to be irritant in a primary skin irritation study undertaken in the rabbit and deemed to cause irreversible eye damage in the eye irritancy test in the rabbit, which was deemed sufficient for classification.

Two skin sensitisation studies have been undertaken, each employing different methodologies: Buehler and a modified LLNA. The Buehler assay returned a negative result, however it is recognised that the Buehler methodology is less sensitive than the maximization or LLNA test. In the modified LLNA, under the evaluation criteria detailed in the study report, Spiroxamine EC 500 (500 g/L) was deemed to be a skin sensitiser. This evaluation criteria was based on a combination of absolute lymph node cell counts, lymph node weights and ear swelling. Collectively, the data generated do not fully follow either OECD 429 or OECD 442B test guideline, with the data only providing supplementary information. However, the active ingredient, spiroxamine, is confirmed to be a skin sensitiser in studies using the Buehler method and the Maximization method (CA 5.2.6/01 [M-016682-01-1] and CA 5.2.6/02 [M-006309-01-1], respectively). Therefore, in accordance with Annex I for Regulation (EC) 1272/2008, as the generic concentration limit of the ingredient within the formulation exceeds the trigger level of 0.1%, Spiroxamine EC 500 (500 g/L) is classified as Skin Sensitisation Category 1, H317 (may cause an allergic skin reaction).

Therefore, Spiroxamine EC 500 (500 g/L) does not warrant classification for dermal toxicity, with classification required for acute oral (Acute Tox. Cat. 4, H302), acute inhalation (Acute Tox. Cat. 4,

H332), skin irritancy (Skin Corrosion/Irritation, Cat. 2, H315), irreversible eye damage (Serious eye Damage/Irritation, Cat. 1, H318) and skin sensitisation (Skin Sensitisation, Cat. 1, H317) according to the harmonised classification Regulation 1272/2008.

Table CP 7.1-1: Acute toxicity studies with Spiroxamine EC 500 (500 g/L)

Type of study	Species	Results	Classification (Annex I for Regulation (EC) 1272/2008)	Annex CA Point / Reference
Oral route	Rat	LD_{50} ♂: 1000 mg/kg bw LD_{50} ♀: >200 – <1000 mg/kg bw	Acute Tox. Cat. 4, H302	CP 7.1.1/01 M-016267-01-1
	Rat	LD_{50} ♂/♀: >500 mg/kg bw		CP 7.1.1/02 M-016680-01-1
Dermal route	Rat	LD_{50} ♂/♀: >2000 mg/kg bw	Insufficient for classification	CP 7.1.2/01 M-016278-01-1
Inhalation route	Rat	LD_{50} 4 h ♂/♀: 2.323mg/L (418.1 mg/kg bw)	Acute Tox. Cat. 4, H332	CP 7.1.3/01 M-030052-01-1
Skin irritation	Rabbit	Erythema was not reversible by day 14	Skin Corrosion/Irritation Cat. 2, H315	CP 7.1.4/01 M-008080-01-1
Eye irritation	Rabbit	Irreversible eye damage	Serious eye Damage/Irritation, Cat. 1, H318	CP 7.1.5/01 M-008080-01-1
Skin sensitisation	Guinea pig	Skin sensitiser (Buehler method)	Test methodology insufficient to conclude on classification and labelling	CP 7.1.6/01 M-006326-01-1
Skin sensitisation	Mouse	Skin sensitiser (modified LLNA)	Skin Sensitisation, Cat. 1, H317	CP 7.1.6/02 M-303647-01-1

CP 7.1.1 Oral toxicity

Data Point:	KCP 7.1.1/01
Report Author:	[redacted]
Report Year:	1994
Report Title:	KWG 4168 500 EC 04023/0021 - Study on the acute oral toxicity in rats
Report No:	22956
Document No:	M-016267-01-1
Guideline(s) followed in study:	US-EPA Series 81-1; OECD 401
Deviations from current test guideline:	Yes OECD 401 has been deleted and is superseded today by OECD 420/423/425, the newer guidelines provide sufficient information on the relevant endpoint (oral LD50) by using less test animals than OECD 401 However, the results produced by OECD 401 are still valid.
Previous evaluation:	yes, evaluated and accepted DAR (1999), 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 KWG 4168 500 EC (Spiroxamine 500 g/L EC) was investigated in a study in the rat performed to GLP and OECD 401 (1987). Groups of Wistar rats (5/sex) received a single oral gavage dose of Spiroxamine 500 g/L EC at dose levels of 100, 200, 1000 and 2000 mg/kg bw for male rats and 100, 200 and 1000 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.

Deaths occurred within 2-3 hours of dosing at dose level 1000 mg/kg bw (2M, 5F) and within 2 days of dosing at a dose level of 2000 mg/kg bw (4M). Signs of toxicity were observed at dose levels of 200 mg/kg bw and above which were reflective of CNS type effects (including but not limited to piloerection, apathy, decreased motility, staggering gait, spastic gait, extended legs, temporary rolling over, lateral position, spasmodic state, temporary chewing movements). All rats gained weight over the study period.

Gross necropsy revealed the following changes in animals which died during the post-treatment observation period: slightly deflated and partly spotty lungs, discoloured and pale spleen, discoloured and dark red liver and compound remnants, change of contents, liquid and mucus in the stomach. 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 Spiroxamine 500g/L EC was calculated to be ~1000 mg/kg bw in male rats and >200 to <1000 mg/kg bw in female rats. Therefore, according to Annex I for Regulation (EC) 1272/2008, Spiroxamine 500 g/L EC is classified as Acute Toxicity (Oral) Category 4, H302 (harmful if swallowed).

Materials and methods

A. Materials:

1. **Test Material:** Spiroxamine 500 g/L EC
(alternative name: KWG 4168 500 EC; KWG 4168 EC 500 04023/0021)

Description:	Clear yellow liquid
Lot/Batch No.:	04023/0021
Purity:	494 g spiroxamine/L
CAS No.:	118134-30-8 (active ingredient)
Stability of test compound:	Confirmed stable for the duration of the study (expiry date: 17 September 1993)

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

3. **Test animals:**

Species:	Rat
Strain:	Wistar (SPF, Hsd/Win:Wu)
Age at dosing:	♂: 7-8 wks; ♀: 10-12 wks
Weight at dosing:	♂: 165-186 g; ♀: 175-191 g
Source:	[redacted]
Acclimation period:	At least 5 days
Diet:	Altromin 1324 Diet for Rats and Mice, *ad libitum* (except for 16-18 h before and 2 h after dosing)
Water:	Municipal water, *ad libitum*
Housing:	Group housed (5/sex/cage) during acclimatisation, singly housed during study phase

4. **Environmental**

conditions:

Temperature:	21 ± 1.5ºC
Humidity:	40 ± 70%
Air changes:	*ca*. 10/h
Photoperiod:	*ca.* 12 h light/dark cycle

B. Study Design:

1. In life dates:	2 June 1993 to 8 July 1993 (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*. 16-18 hours, rats (5/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: ♂: 100, 200, 1000 and 2000 mg/kg bw; ♀: 100, 200 and 1000 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 Days 1 (prior to dosing), 4, 8 and 15.
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 reflective of CNS toxicity were observed in both sexes between 10 minutes and 2 days of dosing at dose levels of 200 mg/kg bw and above. These included apathy, piloerection, laboured breathing, increased salivation, red coloured salivation, red secretion around the eyes, protruding eyes, narrowed palpebral fissure, reduced motility, staggering gait, spastic gait, extended legs, temporary rolling over, lateral position, spasmodic state and temporary chewing movements.
2. Mortality:	Mortalities were observed in all ♀ within 5 h of dosing at 1000 mg/kg bw. Mortalities were observed in 2 ♂ within 2 h of dosing at 1000 mg/kg bw and in four ♂ within 2 days of dosing at 2000 mg/kg bw.

C. Body weight and food consumption:

1. Body weight:	Body weight gain was reduced in ♂ who received 1000 mg/kg bw. With 4/5 ♂ in the 2000 mg/kg bw and all ♀ in the 1000 mg/kg bw dosage group dying on day 1 of dosing, body weight gain assessment could not be performed (refer to Table CP 7.1.1/01-1 and Table CP 7.1.1/01-2)

2. Food consumption: Not measured.

Table CP 7.1.1/01-1-: Overview of acute oral toxicity in male rats treated with Spiroxamine EC 500 (500 g/L): mortality and body weight

Parameter	Dose level (mg/kg bw)															
	100				200				1000				2000			
Overall mortality[a]	0/5				0/5				2/5				4/5			
Day	1	4	8	15	1	4	8	15	1	4	8	15	1	4	8	15
Mortality[a]	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	2/5	0/5	0/5	0/5	4/5	0/1	0/1	0/1
Body weight (g) ±s.d	176 ±6.2	206 ±6.1	227 ±9.3	257 ±15.4	171 ±5.2	201 ±4.6	224 ±9.4	251 ±13.1	186 ±4.6	191 ±6.0	215 ±5.3	245 ±9.0	173 ±2.2	178 ±n.a	208 ±n.a	254 ±n.a
Net body weight gain (g)	80.6 ±11.0				80.4 ±9.7				63 ±9.5				80 ±n.a			
Acute oral LD_{50}	1000 mg/kg bw															

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

Table CP 7.1.1/01-2-: Overview of acute oral toxicity in female rats treated with Spiroxamine EC 500 (500 g/L): mortality and body weight

Parameter	Dose level (mg/kg bw)											
	100				200				1000			
Overall mortality[a]	0/5				0/5				5/5			
Day	1	4	8	15	1	4	8	15	1	4	8	15
Mortality[a]	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	5/5	-	-	-
Body weight (g) ±s.d	181 ±5.5	194 ±8.5	201 ±8.8	205 ±9.2	178 ±2.0	197 ±3.7	200 ±3.9	200 ±2.8	180 ± 2.6	-	-	-
Net body weight gain (g)	23 ±5.6				22 ±2.8				n.a ±n.a			
Acute oral LD_{50}	>200 to <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 slightly deflated and partly spotty lungs, discoloured and pale spleen, discoloured/dark red liver, and test article remnants, change of contents, liquid and mucus in the stomach. Animals sacrificed at the end of the post-treatment observation period showed no evidence of test article-related gross pathological changes.

E. Deficiencies:

OECD 401 has been deleted and is superseded by OECD 420/423/425, the newer guidelines provide sufficient information on the relevant endpoint (oral LD_{50}) by using less test animals than OECD 401. However, the results produced by OECD 401 are still valid.

Conclusions

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 Spiroxamine 500g/L EC was calculated to be ~1000 mg/kg bw in male rats and >200 to <1000 mg/kg bw in female rats. Therefore, according to Annex I for Regulation (EC) 1272/2008, Spiroxamine 500g/L EC is classified as Acute Toxicity (Oral) Category 4, H302 (harmful if swallowed).

Data Point:	KCP 7.1.1/02
Report Author:	[redacted]
Report Year:	1998
Report Title:	KWG 4168 500 EC 04023/0626 - Study for acute oral toxicity in rats
Report No:	27457
Document No:	M-016680-01-1
Guideline(s) followed in study:	OECD 401; US-EPA Series 81-1, Directive 67/548/EEC, Annex V, Part B.1.
Deviations from current test guideline:	Yes OECD 401 has been deleted and is superseded today by OECD 420/423/425, the newer guidelines provide sufficient information on the relevant endpoint (oral LD50) by using less test animals than OECD 401 However, the results produced by OECD 401 are still valid.
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 KWG 4168 500 EC (Spiroxamine 500 g/L EC) was investigated in a study in the rat performed to GLP and OECD 401 (1987). Groups of Wistar rats (5/sex) received a single oral gavage dose of Spiroxamine 500 g/L EC at a dose level of 500 mg/kg bw and were observed for 14 days. The test article was formulated in demineralised water and administered oral via gavage employing a dose volume of 10 mL/kg bw.

No mortalities were observed throughout the study period, with all animals surviving to the scheduled sacrifice. Clinical signs reflective of CNS toxicity were observed in both sexes between 20 minutes and 6 hours of dosing at 500 mg/kg bw. These included, but were not limited to decreased motility and

reactivity, staggering and uncoordinated gait, spasmodic state and laboured breathing. All animals gained weight during the study period.

Gross necropsy confirmed no evidence of test article related gross organ lesions.

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

Materials and methods

A. Materials:

1. Test Material:	Spiroxamine 500 g/L EC (alternative name: KWG 4168 500 EC; KWG 4168 EC 500; 04023/0021)
Description:	Clear brown liquid
Lot/Batch No.:	233725201
Purity:	505 g spiroxamine/L
CAS No.:	118134-30-8 (active ingredient)
Stability of test compound:	Confirmed stable for the duration of the study (expiry date: 19 November 1998)
2. Vehicle and/or positive control:	Demineralised water; not applicable
3. Test animals:	
Species:	Rat
Strain:	Wistar (SPF, HsdCpb:Wu)
Age at dosing:	♂: 6-7 wks; ♀: 8 wks
Weight at dosing:	♂: 179-201 g; ♀: 169-176 g
Source:	[redacted]
Acclimation period:	At least 5 days
Diet:	Altromin 1324 Diet for Rats and Mice, *ad libitum* (except for *ca*. 17 h before and 2 h after dosing)
Water:	Municipal water, *ad libitum*
Housing:	Group housed (5/sex/cage) during acclimatisation, singly housed during study phase
4. Environmental conditions:	
Temperature:	22 ± 2°C
Humidity:	55 ± 5%
Air changes:	*ca*. 15-20/h
Photoperiod:	12 h light/dark cycle

B. Study Design:

1. In life dates:	13 January to 28 January 1998 (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 (5/sex/gp) were administered the test article by single oral *via* gavage, employing a dose volume of 10 mL/kg bw, for the following doses: ♂/♀: 500 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 was 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 Days 1 (prior to dosing), weekly 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 between 20 minutes and 6 hours of dosing at 500 mg/kg bw. These included decreased motility and reactivity, staggering and uncoordinated gait, spasmodic state and laboured breathing. Additionally, temporary rolling over was observed in ♂ and increased salivation was observed in ♀.

2. Mortality: No unscheduled deaths were observed, with all animals surviving to the scheduled necropsy.

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 7.1.1/02-1.

2. Food consumption: Not measured.

Table CP 7.1.1/02-1-: Overview of acute oral toxicity in rats treated with Spiroxamine EC 500 (500 g/L): mortality and body weight

Parameter	Dose level (mg/kg bw)					
	♂ (500)			♀ (500)		
Overall mortality[a]	0/5			0/5		
Day	1	8	15	1	8	15
Body weight (g) ±s.d	188 ±8.2	246 ±10.2	278 ±19.6	171 ±2.8	201 ±1.9	213 ±6.7
Net body weight gain (g)	89 ±15.8			42 ±5.2		
Acute oral LD_{50}	>500 mg/kg bw			>500 mg/kg bw		

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

D. Necropsy:

Animals sacrificed at the end of the post-treatment observation period revealed no evidence of test article related gross organ lesions.

E. Deficiencies:

OECD 401 has been deleted and is superseded today by OECD 420/423/425, the newer guidelines provide sufficient information on the relevant endpoint (oral LD_{50}) by using less test animals than OECD 401, but not because OECD 401 would have produced less valid results.

Conclusions

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 Spiroxamine 500 g/L EC was calculated to be >500 mg/kg bw in male and female rats. Therefore, according to Annex I for Regulation (EC) 1272/2008, Spiroxamine 500 g/L 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:	1994
Report Title:	KWG 4168 500 EC 04023/0921 - Study on the acute dermal toxicity in rats
Report No:	22957
Document No:	M-016278-01-1
Guideline(s) followed in study:	US-EPA Series 81-2, OECD 402
Deviations from current test guideline:	Yes Although the study was conducted according to test guideline OECD 402 (1987), this test guideline has since been updated in the intervening period (2017). When assessed against current test guideline requirements the following deficiencies are noted: Updated guideline requires 2 animals of the more sensitive sex in each dose level. Dose levels that are fixed and equal to 50, 200, 1000 and 2000 mg/kg bw, as an initiating dose. The test article was held in place with an occlusive dressing, rather than the recommended semi-occlusive dressing. This represents a worst case scenario, and does not invalidate the study. The results attained through the previous guideline are still valid.
Previous evaluation:	yes, evaluated and accepted DAR (1999), 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 KWG 4168 500 EC (Spiroxamine 500g/L EC) was investigated in a study in rats performed to GLP and OECD 402 (1987). Spiroxamine 500g/L EC (mixed with 400 mg cellulose powder/g formulation) was applied to the shorn dorsal skin of Wistar rats (5/sex/group) at dose levels of 100, 500 and 2000 mg/kg bw (both sexes). Rats were observed for a period of 14 days.

Local effects at the site of application were evident from 500 mg kg/bw, with reddening/encrustation/hardening/scars/wrinkling/dark discolouration/squamae from 2 hours post-dose; these were largely reversible during the observation period. Signs of systemic toxicity in both sexes were observed in rats administered dose levels of 500 mg/kg bw and above which included red secretion and encrustation around the snout and eyes. In females only, evidence of systemic toxicity manifest as

CNS type effects was reported and included (but not restricted to) apathy, piloerection, pallor, laboured breathing, increased salivation, narrowed palpebral fissure, staggering gait, spastic gait, extended hind legs, spasmodic state, uncoordinated motions, and decreased motility. Findings were observed from 3 hours post-dose. Body weight gains were impaired on study Day 4 in all test groups, however these had returned to normal by the end of the study. Mortalities were observed in 2/5 females 3 days post dosing at 2000 mg/kg bw.

Gross necropsy of decedents revealed abnormalities including slightly deflated lungs, discolouration and spotting of the stomach, discolouration of the kidneys and reddening, change of contents, empty, red and mucous of the intestine. Of the animals sacrificed at the end of the post-treatment observation period, one male was observed with reduced in size testicles. No other animal sacrificed at the end of the post-treatment observation period showed evidence of test article-related gross pathological changes.

Under the conditions of this study, the acute dermal LD_{50} of Spiroxamine 500 g/L EC was found to be >2000 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.

Materials and methods

A. Materials:

1. Test Material:	Spiroxamine 500 g/L EC (alternative name: KWG 4168 500 EC; KWG 4168 EC 500-04023/0021)
Description:	Clear yellow liquid
Lot/Batch No.:	04023/0021
Purity:	494.0 g/L
CAS No.:	118134-30-8 (active ingredient)
Stability:	Confirmed stable for the duration of the study (expiry date: 17 September 1993)
2. Vehicle and/or positive control:	0.4% (w/w) cellulose/not applicable
3. Test animals:	
Species:	Rat
Strain:	Wistar (SPF Hsd/Win.Wu)
Age at dosing:	♂: 9-12 wks; ♀: >16 wks (age based on body weight)
Weight at dosing:	♂: 213-290 g; ♀: 218-249 g
Source:	[redacted]
Acclimation period:	At least 5 days
Diet:	Altromin 1324 Diet for Rats and Mice, *ad libitum*
Water:	Municipal water, *ad libitum*
Housing:	Group housed (5/sex/cage) during acclimatisation, singly housed during study phase
4. Environmental conditions:	
Temperature:	21±1.5ºC
Humidity:	55±5%
Air changes:	At least 10/h
Photoperiod:	*ca* 12 light/dark cycle

B. Study Design:

1. In life dates:	2 June 1993 to 07 July 1993 (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 5.5 *x* 5.5 cm). On the day of application, Spiroxamine 500 EC was mixed with cellulose powder and applied evenly to the pre-clipped dorsal skin at various dose levels to 5 rats/sex/group: 100, 500 and 2000 mg/kg bw. The ♂ received 1.1 mg test article/cm^2 in the 100 mg/kg bw dose group and 20.2 – 23.2 mg test article/cm^2 in the 2000 mg/kg bw dose group. The ♀ received 1.2 mg test article/cm^2 in the 100 mg/kg bw dose group and 18.5 – 19.2 mg test article/cm^2 in the 2000 mg/kg bw dose group. The test article was weighed and mixed on aluminium foil (comparable in size to the shaved test site), which was then applied to the test site and secured in place with a bandage dressing. After 24 hours exposure, the dressings were removed and the treated skin site cleaned with soap and water. 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 was recorded several times on the day of treatment, and at least once a day thereafter for 14 days.
3. Body weights:	Recorded on Study Days 1 (prior to dosing), 4, 8 and 15 post dosing.
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 at the site of application were evident from 500 mg/kg bw with reddening/encrustation/hardening/scars/wrinkling/dark discolouration/squamae from 2 hours post-dose, these were largely reversible during the observation period. Signs of toxicity in both sexes were observed in rats administered dose levels of 500 mg/kg bw and above which included red secretion and encrustation around the snout and eyes. In ♀ only, evidence of systemic toxicity manifest as CNS type effects were reported and included apathy, piloerection, pallor, laboured breathing, increased salivation, narrowed palpebral fissure, encrustation at the labial commissure, staggering gait, spastic gait, extended hind legs, spasmodic state, uncoordinated motions, decreased motility, lateral position and lateral position of the head. Findings were observed from 3 hours post-dose.
2. Mortality:	Mortalities were observed in 2/5 ♀ 3 days post dosing at 2000 mg/kg bw. Refer to Table CP 7.1.2/01-01 and Table CP 7.1.2/01-2

Table CP 7.2.1/01-1-: Overview of acute dermal toxicity in male rats treated with Spiroxamine EC 500 (500 g/L): mortality and body weight

Parameter	Dose level (mg/kg bw)											
	100				500				2000			
Overall mortality[a]	0/5				0/5				0/5			
Day	1	4	8	15	1	4	8	15	1	4	8	15
Body weight (g) ±s.d	220 ±5.5	228 ±8.4	250 ±13.3	284 ±19.3	251 ±8.6	252 ±11.4	265 ±13.1	295 ±17.9	274 ±14.9	256 ±13.8	280 ±15.5	320 ±26.0
Net body weight gain (g)	64 ±14.1				44 ±14.0				46 ±12.3			
Acute oral LD_{50}	>2000 mg/kg bw											

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

Table CP 7.1.2/01-2-: Overview of acute dermal toxicity in female rats treated with Spiroxamine EC 500 (500 g/L): mortality and body weight

Parameter	Dose level (mg/kg bw)											
	100				500				2000			
Overall mortality[a]	0/5				0/5				2/5			
Day	1	4	8	15	1	4	8	15	1	4	8	15
Mortality[a]	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	2/5	0/3	0/3	0/3
Body weight (g) ±s.d	242 ±4.2	242 ±8.6	250 ±8.1	256 ±9.4	234 ±12.5	225 ±12.6	231 ±13.3	245 ±14.4	235 ±4.1	218 ±11.7	229 ±4.7	239 ±6.4
Net body weight gain (g)	13 ±6.6				12 ±5.2				3 ±2.1			
Acute oral LD_{50}	>2000 mg/kg bw[b]											

a Mortality: no. of animals found dead / no. of animals treated
b prudent to acknowledge that the report stated LD_{50} was <2000 mg/kg bw. However as only 2/5/5 ♀ at this dose died, LD_{50} >2000 mg/kg bw

C. Body weight and food consumption:

1.Body weight:	Body weight gains were impaired on study Day 4 in all test groups, however these had returned to normal by the end of the study.
2.Food consumption:	Not measured.

D. Necropsy:

Gross necropsy of decedents revealed abnormalities including slightly deflated lungs, discolouration and spotting of the stomach, discolouration of the kidneys and reddening, change of contents, empty, red and mucous of the intestine. Of the animals sacrificed at the end of the post-treatment observation period, one male was observed with reduced in size testicles. No other animal sacrificed at the end of the post-treatment observation period showed evidence of test article-related gross pathological changes.

E. Deficiencies:

Although the study was conducted according to test guideline OECD 402 (1987), this test guideline has since been updated in the intervening period (2017). When assessed against current test guideline requirements the following deficiencies are noted:

Updated guideline requires 2 animals of the more sensitive sex in each dose level.

The test article was held in place with an occlusive dressing, rather than the recommended semi-occlusive dressing. This represents a worst case scenario, and does not invalidate the study.

The results attained through the previous guideline are still valid.

Conclusions

> <u>**Assessment and conclusion by applicant:**</u>
>
> **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 Spiroxamine 500g/L EC was found to be >2000 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:	2000
Report Title:	KWG 4168 500 EC 04023/0626 (c.n.: Spiroxamine) - Study on acute inhalation toxicity in rats according to OECD no. 403
Report No:	29759
Document No:	M-030052-01-1
Guideline(s) followed in study:	OECD 403; Directive 92/69/EEC, Method B.2.; US-EPA 712C-98-193, OPPTE 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 Spiroxamine 500 g/L EC was investigated. 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 1033 and 5225 mg/m^3, with MMAD ±GSD of 1.41 ±2.56 and 1.74 ±2.23, respectively obtained for the aerosol size distribution, with >75% of the particles within 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 1033 mg/m^3 and above. For surviving animals, all were free of clinical signs from day 10 (males) or 6 (females) of the post-treatment observation period. Statistically significant reductions in rectal temperature were observed in animals from the 1033 mg/m^3 dose groups. As animals treated at 5225 mg/m^3 died during the exposure, no assessment of body temperature could be made.

Deaths occurred during the study at a concentration of 5225 mg/m^3 with death occurring during exposure.

The rats exposed to Spiroxamine 500 g/L EC concentrations of 1033 mg/m^3 and above experienced a transient effect on bodyweight over the post-treatment period. Gross necropsy revealed that animals which were sacrificed at the end of the observation period showed no evidence of treatment-related changes in the lungs or other organs, although animals that had died during the study had multiple changes to the lungs, liver and spleen.

Under the conditions of this study the rat acute inhalation 4 hour nose only LC_{50} of Spiroxamine 500 g/L EC is 2.323 mg/L in males and females (equivalent to 418.1 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:	Spiroxamine 500 g/L EC (alternative name: KWG 4168 500 EC ; KWG 4168 EC 500 04023/0021)
Description:	Translucent (clear), brownish liquid
Lot/Batch No.:	23372520I
Purity:	507 g/L
CAS No.:	118134-30-8
Stability of test compound:	Confirmed stable for the duration of the study (expiry date: 12 March 2000)
2. Vehicle and/or positive control:	None / not relevant
3. Test animals:	
Species:	Rat
Strain:	Hsd CpB:WU (SPF)
Age at dosing:	*ca.* 8 wks
Weight at dosing:	♂: 162-210g; ♀: 164-184g
Source:	[redacted]
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:	22 November 1999 to 13 December 1999 (experimental dates)
2. Animal assignment and treatment:	Following acclimatisation rats were randomly assigned to the test groups. Groups of rats (5/sex) were exposed (nose only) for 4 hours to atmospheres containing Spiroxamine 500 g/L EC (aerosol) at gravimetric concentrations of 0 (vehicle control), 1033 or 5225 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). Spiroxamine 500 g/L EC 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 spiroxamine in the test atmosphere was performed using gas chromatography (FI detector). Temperature and air humidity in the exposure chamber were measured over 10 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:	Rats were observed 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 Spiroxamine EC 500 (500 g/L): exposure parameters of the acute inhalation toxicity

Parameter	Value	
Dose group (nominal mg/m^3)	1000	5000

Parameter		Value	
Mean achieved atmosphere concentration (mg/m^3)		1033	5225
Mean achieved atmosphere concentration (mg/L)		1.033	5.225
Dose group (internal dose mg/kg bw/d)[a]		185.9	940.5
Chamber flow rate (L/min)		15	15
Particle size (MMAD ± GSD)		1.41 ±2.56	1.74 ±2.23
Aerosol mass <3 µm (%)		78.9	75.1
Chamber air temperature (°C)	During exposure	21	21
Relative humidity (%)	During exposure	Not detailed	
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:

<u>0 mg/m³</u>: no clinical signs of toxicity were evident.

<u>1033 mg/m³ (185.9 mg/kg bw)</u>: clinical signs of toxicity manifest as CNS type effects were reported (including piloerection and ungroomed fur, bradypnea, labour breathing, reduced motility, nasal discharge, nostrils with red encrustations, pallor, tremors, rales).

<u>5225 mg/m³ (940.5 mg/kg bw)</u>: all animals died during exposure, therefore no clinical signs were reported.

For surviving animals, all were free of clinical signs from day 10 (♂) or 6 (♀) of the post-treatment observation period.

Reflex examination performed post exposure indicated no evidence of an influence on reflexes in treated animals.

2. Mortality

Deaths were restricted to animals dosed at 5225 mg/m³ (5♂, 5♀), all occurring within the 4 hour exposure period.

Refer to Table CP 7.1.3/01-1.

3. Rectal temperatures:

Statistically significant reductions in rectal temperature were observed in animals from the 1033 mg/m³ dose groups. As animals treated at 5225 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 Spiroxamine EC 500 (500 g/L): mortality and body weight

Parameter	♂ (actual concentration (mg/m³) [target mg/m³])												♀ (actual concentration (mg/m³) [target mg/m³])											
	0				1033 [1000]				5225 [5000]				0				1033 [1000]				5225 [5000]			
Overall mortality[a]	0/5				0/5				5/5				0/5				0/5				5/5			
Day	0	3	7	15	0	3	7	15	0	3	7	15	0	3	7	15	0	3	7	15	0	3	7	15
Mortality[a]	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	5/5	-	-	-	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	5/5	-	-	-
Body weight (g) ±s.d	171.8 ±6.4	184.2 ±6.7	208.2 ±7.9	237.8 ±7.7	200.6 ±9.3	176.4 ±10.6	205.6 ±12.1	246.8 ±18.5	175.6 ±1.7	-	-	-	168.2 ±4.6	173.2 ±6.5	180.2 ±4.4	186.4 ±4.7	178.2 ±6.0	164.2 ±5.6	177.0 ±4.2	186.2 ±6.7	175.8 ±2.6	-	-	-
Net body weight gain (g)	66 ±4.9				46 ±10.1				-				18 ±3.4				8 ±2.8				-			
Rectal temp. (°C) at end of treatment	37.6				31.1**				-				38.1				29.7**							
Acute oral LC_{50}	2323mg/m³ (2.323 mg/L), equivalent to 418.1 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 4 in animals from the 1033 mg/m^3, with recovery thereafter. As animals treated at 5225 mg/m^3 died during the exposure, no assessment of body weight gain could be made.
2. Food consumption:	Not measured

D. Necropsy:

Animals which died during exposure had evidence of lung oedema, foamy content in trachea, discharge of clear liquid nose, hydrothorax (pleural effusion), focal discolorations. Liver lobulation and pale spleen were evident.

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

Conclusions

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} of 500 g/L EC is 2.323 mg/L in males and females (equivalent to 418.1 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:	1992
Report Title:	KWG 4168 EC 00500 04023/0021 - Study for skin and eye irritation/corrosion in rabbits
Report No:	21260
Document No:	M-008080-01-1
Guideline(s) followed in study:	OECD 405, Directive 84/449/EEC, Method 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 dermal irritation study, 3 female New Zealand White rabbits were dermally exposed to 0.5 mL of Spiroxamine 500 g/L EC 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, and 72 hours, 7 and 14 days after patch removal with erythema/eschar and oedema formation scored after patch removal. Irritation was scored according to the assessment criteria for primary skin irritation (Draize scale). The skin irritation/corrosion test was repeated with the test article applied at 1% and 10% (diluted in deionised water).

Spiroxamine 500 g/L EC applied at 1%: a single animal displayed very slight erythema (grade 1) at patch removal. At the 24 hour time point and onwards, no signs of irritation were observed in the three animals treated, with observations terminated at day 7.

Spiroxamine 500 g/L EC applied at 10%: signs of irritation, limited to erythema were observed in all three rabbits from the 1-hour observation, with grade 1 observations noted. From the 24 hour time point erythema had increased to grade 2 in 2/3 animals, with the other animal exhibiting grade 1 erythema. These effects continued through to day 7, with 1/3 animals exhibiting grade 1 erythema and with slight eschar formation. By day 14 this animal exhibited no evidence of skin irritation, with 2/3 animals exhibiting squamous white coat.

Spiroxamine 500 g/L EC applied undiluted: signs of irritation were observed in all three rabbits from the 1-hour observation and included erythema (up to Grade 2), with oedema (up to Grade 3) occurring from the 24 hour time point. Dermal reactions (Grade 1 or 2 erythema, Grade 1 or 2 oedema) persisted to the 7-day observation, with erythema (grade 1) still present at the end of the 14-day observation period in two rabbits.

Under the conditions of this study, Spiroxamine 500 g/L EC caused dermal irritation that was not reversible by day 14. Therefore, according to Annex I for Regulation (EC) 1272/2008, Spiroxamine 500g/L EC is classified as Skin Corrosion/Irritation, Category 2, H315 (causes skin irritation).

Materials and Methods

A. Materials:

1. Test Material:	Spiroxamine 500 g/L EC (alternative name: KWG 4168 5EC 00500 EC)
Description:	Clear brownish liquid
Lot/Batch No.:	04023/0021
Purity:	495 g/L (49.5%), pH 9.4 (2% in 0.1% saline)
CAS No.:	118134-30-8 (active ingredient)
Stability of test compound:	Assumed stable for the duration of the study (expiry date not confirmed)
2. Vehicle and/or positive control:	Deionised water/not applicable
3. Test animals:	
Species:	Rabbits
Strain:	New Zealand White
Age at dosing:	Not given, but based on body weight estimated age: 11 – 13 wks
Weight at dosing:	♀: 2.7-3.2 kg
Source:	[redacted]
Acclimation period:	At least 14 days
Diet:	Ssniff K 4, *ca*. 100 - 120 g/animal/day
Water:	Municipal water, *ad libitum*

Housing: Individually housed

4. Environmental conditions:

Temperature: 21 ±1.5°C
Humidity: 55 ±15%
Air changes: 12-15/h
Photoperiod: 12 hour light/dark

B. Study Design:

1. In life dates: 14 January 1992 to 4 February 1992 (experimental dates)

2. Animal assignment and treatment: Animals were allocated by random sampling. 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 a hypoallergenic patch and another patch was moistened with water. These patches were then applied on opposite dorso-lateral areas of the trunk of each animal. 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, and 72 hours, 7 and 14 days after patch removal with erythema/eschar and oedema formation scored.

The skin irritation/corrosion test was repeated with the test article applied at 1% and 10% (diluted in deionised water).

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 at 7 and 14 days.

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:	Spiroxamine 500 g/L EC applied at 1%: a single animal displayed very slight erythema (grade 1) at patch removal. At the 24 hour time point and onwards, no signs of irritation were observed in the three animals treated, with observations terminated at day 7. Spiroxamine 500 g/L EC applied at 10%: signs of irritation, limited to erythema were observed in all three rabbits from the 1-hour observation, with grade 1 observations noted. From the 24 hour time point erythema had increased to grade 2 in 2/3 animals, with the other animal exhibiting grade 1 erythema. These effects continued through to day 7, with 1/3 animals exhibiting grade 1 erythema and with slight eschar formation. By day 14 this animal exhibited no evidence of skin irritation, with 2/3 animals exhibiting squamous white coat. Spiroxamine 500 g/L EC applied undiluted: signs of irritation were observed in all three rabbits from the 1-hour observation and included erythema (up to Grade 2), with oedema (up to Grade 3) occurring from the 24 hour time point. Dermal reactions (Grade 1 or 2 erythema, Grade 1 or 2 oedema) persisted to the 7-day observation, with erythema (grade 1) still present at the end of the 14-day observation period in two rabbits.

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

Animal no.	Erythema						Oedema					
	Time point											
	1 h	24 h	48 h	72 h	8 d	14d	1 h	24 h	48 h	72 h	8 d	14d
Spiroxamine 500 g/L EC applied at 1%												
L8	0	0	0	0	0	-	0	0	0	0	0	-
M18	0	0	0	0	0	-	0	0	0	0	0	-
M19	0	0	0	0	0	-	0	0	0	0	0	-
Mean (24 – 72 h)	0.0						0.0					
Spiroxamine 500 g/L EC applied at 10%												
M4	1	2	1	1	0	0	0	0	0	0	0	0[a]
L5	1	1	1	1	0	0	0	0	0	0	0	0[a]
K4	1	2	2	2	1	0	0	0	0	0	0[b]	0
Mean (24 – 72 h)	1.4						0.0					
Spiroxamine 500 g/L EC applied undiluted												
M15	1	2	2	3	2[c]	1[d]	0	1	2	2	1[e]	0[d]
M1	2	2	2	2	1[c]	0[d]	0	1	2	2	1[e]	0[d]
K16	2	2	3	3	3[e]	1[d]	0	1	1	1	1[e]	0[d]

Animal no.	Erythema						Oedema					
	Time point											
	1 h	24 h	48 h	72 h	8 d	14d	1 h	24 h	48 h	72 h	8 d	14d
Mean (24 – 72 h)	2.3						1.4					

- not examined
a white squamous coat
b slight eschar formation
c hardening of the skin
d exposed skin area: loss of hair
e eschar formation

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, Spiroxamine 500 g/L EC caused dermal irritation that was not reversible by day 14. Therefore, according to Annex I for Regulation (EC) 1272/2008, Spiroxamine 500g/L EC 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:	1992
Report Title:	KWG 4168 EC 00500 04023/0021 - Study for skin and eye irritation/corrosion in rabbits
Report No:	21260
Document No:	M-008080-01-1
Guideline(s) followed in study:	OECD 405; Directive 84/449/EEC, Method 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 Spiroxamine 500 g/L EC was instilled into the left eye of 3 female New Zealand White rabbits. Eyelids were held to together for ~1 second to prevent loss of material. The other eye served as a control. After 24 hours, the treated eye was rinsed with saline. Both eyes of each animal were observed at 1, 24, 48, 72 hours, 8, 14 and 21 days after application. Changes to the cornea, iris and conjunctiva were observed periodically by the Draize method. The eye irritation/corrosion test was repeated with the test article applied at 1% and 10% (diluted in deionised water).

Spiroxamine 500 g/L EC applied at 1%: grade 1 redness of the iris, (1/3 animals), conjunctival erythema (3/3) and conjunctival chemosis (3/3) were observed, with complete reversal by observation day 7.

Spiroxamine 500 g/L EC applied at 10%: grade 1 corneal opacity was observed in all animals, completely resolving by observation day 7. Iris redness results in 1/3 animals with grade 1 observation at 1 hour post instillation only. Grade 2 conjunctival erythema was observed in all animals, completely resolving by day 14. Grade 3 conjunctival chemosis was observed in all animals, completely resolving by day 14

Spiroxamine 500 g/L EC applied undiluted: corneal opacity, conjunctival erythema and chemosis achieved grade 3 scores, not resolving by day 21. Iris redness in 2/3 animals was not scorable by day 21 in 2/3 animals due to strong corneal opacity.

Under the conditions of this study the test article, Spiroxamine 500 g/L EC showed irreversible eye damage. According to Annex I for Regulation (EC) 1272/2008, Spiroxamine 500g/L EC is classified as Serious eye Damage/Irritation, Category 1, H318 (causes serious eye damage).

Materials and Methods

A. Materials:

1. Test Material: Spiroxamine 500 g/L EC
(alternative name: KWG 4168 5EC 00500 EC)

Description: Clear brownish liquid

Lot/Batch No.:	04023/0021
Purity:	495 g/L (49.5%), pH 9.4 (2% in 0.1% saline)
CAS No.:	118134-30-8 (active ingredient)
Stability of test compound:	Assumed stable for the duration of the study (expiry date not confirmed)
2. Vehicle and/or positive control:	Deionised water/not applicable
3. Test animals:	
Species:	Rabbits
Strain:	New Zealand White
Age at dosing:	Not given, but based on body weight estimated age: 12 - 14 wks
Weight at dosing:	♀: 2.9-3.5 kg
Source:	[redacted]
Acclimation period:	At least 14 days
Diet:	Ssniff K 4, *ca.* 100 - 120 g/animal/day
Water:	Municipal water, *ad libitum*
Housing:	Individually housed
4. Environmental conditions:	
Temperature:	21 ±1.5°C
Humidity:	55 ±15%
Air changes:	12-15/h
Photoperiod:	12 hour light/dark

B. Study Design:

1. In life dates:	14 January 1992 to 4 February 1992 (experimental dates)
2. Animal assignment and treatment:	Animals were allocated into groups by random sampling. The 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 one 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. After 24 hours, the treated eye was rinsed with saline. For each animal, the score on the Draize scale was assigned at 1, 24, 48, 72 hours, 7, 14 and 21 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. The eye irritation/corrosion test was repeated with the test article applied at 1% and 10% (diluted in deionised water).
3. Evaluation criteria:	Eye irritation: <u>Cornea</u> *Opacity: degree of density*: - No ulceration or opacity — 0 - Scattered or diffuse areas of opacity details or 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 thorough 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:	Spiroxamine 500 g/L EC applied at 1%: grade 1 redness of the iris, (1/3 animals), conjunctival erythema (3/3) and conjunctival chemosis (3/3) were observed, with complete reversal by observation day 7. Spiroxamine 500 g/L EC applied at 10%: grade 1 corneal opacity was observed in all animals, completely resolving by observation day 7. Iris redness results in 1/3 animals with grade 1 observation at 1 hour post instillation only. Grade 2 conjunctival erythema was observed in all animals, completely resolving by day 14. Grade 3 conjunctival chemosis was observed in all animals, completely resolving by day 14 Spiroxamine 500 g/L EC applied undiluted: corneal opacity, conjunctival erythema and chemosis achieved grade 3 scores, not resolving by day 21. Iris redness in 2/3 animals was not scorable by day 21 in 2/3 animals due to strong corneal opacity.

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
24 h	0	0	0	0	0	0	1	1	0	0	1	0
48 h	0	0	0	0	0	0	0	0	0	0	1	1
72 h	0	0	0	0	0	0	0	0	0	0	0	1
7 d	0	0	0	0	0	0	0	0	0	0	0	0
Mean (24 – 72 h)	0.0	0.0	0.0	0.0	0.0	0.0	0.3	0.3	0.0	0.0	0.7	0.7
	0.0			0.0			0.2			0.5		
Spiroxamine 500 g/L EC applied at 10%												
1 h	1	1	1	0	0	1	2	2	2	3	3	3
24 h	1	1	1	0	0	0	2	2	2	3	3	3

Time point	Cornea opacity			Iris (redness)			Conjunctival erythema			Conjunctival chemosis		
	Animal number											
	1	2	3	1	2	3	1	2	3	1	2	3
48 h	1	1	1	0	0	0	2	2	2	2	2	2
72 h	1	1	1	0	0	0	2	2	2	1	1	1
7 d	0	0	0	0	0	0	0	0	1	1	0	1
14 d	0	0	0	0	0	0	0	0	0	0	0	0
Mean (24 – 72 h)	1.0	1.0	1.0	0.0	0.0	0.0	2.0	2.0	2.0	3.0	3.0	3.0
	1.0			0.0			2.0			3.0		
Spiroxamine 500 g/L, EC applied undiluted												
1 h	1	1	1	1	0	0	2	2	2	3	3	3
24 h	1	1	1	0	0	0	2	2	2	3	3	3
48 h	1	1	1	1	0	0	3	3	3	4	3	3
72 h	2	2	2	1	1	1	3	3	3	4	3	4
7 d	2	2	2	1	0	1	3	3	3	3	3	3
14 d	2	3	3	0	[a]	[a]	0	2	3	2	3	3
21 d	1	3	3	0	[a]	[a]	0	1	3	2	2	3
Mean (24 – 72 h)	1.3	1.3	1.3	0.6	0.7	0.3	2.7	2.7	2.7	3.7	3.0	3.3
	1.3			0.6			2.7			3.3		

a evaluation not possible due to strong corneal opacity

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.

Conclusion

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, Spiroxamine 500 g/L EC showed irreversible eye damage. According to Annex I for Regulation (EC) 1272/2008, Spiroxamine 500g/L EC is classified as Serious eye Damage/Irritation, Category 1, H318 (causes serious eye damage).

CP 7.1.6 Skin sensitization

Data Point:	KCP 7.1.6/01
Report Author:	[redacted]
Report Year:	1993
Report Title:	KWG 4168 500 EC 04023/0021- Studies on skin sensitizing effect on guinea pigs (Buehler Test)
Report No:	22546
Document No:	M-006326-01-1
Guideline(s) followed in study:	OECD 406; Directive 84/449/EC; US-EPA §81-6
Deviations from current test guideline:	Yes Although the study was conducted according to test guideline OECD 406 (1981), this test guideline has since been updated in the intervening period (1992). When assessed against current test guideline requirements the following deficiencies are 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 argument can be made that the without a concurrent positive control, or historical control data presented the sensitivity and specificity of the test system was not demonstrated at the laboratory. The data generated were sufficient to conclude that spiroxamine is a skin sensitiser, which is confirmed in an independent study employing a different model.
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 3-induction Buehler assay was conducted in guinea pigs (12/group) in order to examine the skin sensitisation potential of spiroxamine. Following a preliminary irritation test, the test article was diluted in physiological saline and initially administered *via* topical application at 12% Spiroxamine 500 g/L EC, applied for 6 hours, with additional inductions 7 and 14 days later. A corresponding control group received physiological saline.

For the challenge, control and treated animals were treated with both the vehicle control (physiological saline) and test article at 3 and 6% *via* topical application for 6 hours. Skin reactions were recorded at 24, 48 and 72 hours after the challenge applications.

After challenge no differences with regard to the incidence and intensity of skin reactions were seen between the test article treated animals and control group animals at both concentrations tested. No dermal reactions occurred in test article treated or control animals following challenge with a non-irritant concentrations of 3 and 6%

Without a concurrent positive control or historical control data, the sensitivity and specificity of the test system was not demonstrated at the laboratory.

Under the conditions of this study the test article, Spiroxamine 500 g/L EC was confirmed to not be a skin sensitiser when examined in the guinea pig employing the Buhler methodology. These data however are considered supplementary as the sensitivity and specificity of the test system at the conducting laboratory had not been demonstrated.

Materials and Methods

A. Materials:

1. Test Material:	Spiroxamine 500 g/L EC (alternative name: KWG 4168 500 EC; KWG 4168 EC 500 04023/0021)
Description:	Clear yellow liquid
Lot/Batch No.:	04023/0021
Purity:	494 g spiroxamine/L
CAS No.:	118134-30-8 (active ingredient)
Stability of test compound:	Confirmed stable for the duration of the study (expiry date: 17 September 1993)
2. Vehicle and/or positive control:	Saline/not included
3. Test animals:	
Species:	Guinea pig
Strain:	BOR:DHPW
Age at dosing:	♂: *ca.* 5 – 8 weeks
Weight at dosing:	♂: 323 – 400g
Source:	[redacted]
Acclimation period:	At least 7 days
Diet:	Altromin® 3020 diet, *ad libitum*
Water:	Municipal water, *ad libitum*
Housing:	Housed 5 animals/cage during acclimatisation, reduced to 3/cage during study phase
4. Environmental conditions:	
Temperature:	21 ±1.5°C
Humidity:	55 ±15°C
Air changes:	*ca.* 12-15/h
Photoperiod:	12 hour light/dark

B. Study Design:

1. In life dates:	25 May 1993 to 25 June 1993 (experimental dates)
2. Preliminary range finder:	A group of 5 albino guinea pigs received four applied concentrations of spiroxamine (0.5 mL) at 0.5, 1, 3, 6, 12, 25, 50 and 100% under occlusive conditions to the left flank for 6 hours. Test sites were depilated prior to application. Dermal reactions were assessed at 24, 48 and 72 hours.
3. Animal assignment and treatment:	Three groups of 12 albino ♂ guinea-pigs of the BOR:DHPW strain were allocated as follows and the Buehler (3 induction) methodology was used to determine the skin sensitisation potential of spiroxamine: 1. Control group: 12 animals 2. Spiroxamine 500 g/L EC: 12 animals 3. Spiroxamine 500 g/L EC: 12 animals *Epicutaneous induction*: 1st induction: 12% 2nd induction: 12% 3rd induction: 12% Treatment sites were depilated the day prior to application. Hypoallergenic dressing containing a 0.5 mL volume were applied for 6 hours, separated by

	7 days and fixed to the skin with adhesive tape. Control animals were treat the same, but with only vehicle. *Topical challenge (3 and 4 weeks after intradermal injections):* Treatment sites (backs and flank) were depilated the day prior to application. The challenges were performed 4 and 5 weeks after the 1st, 2nd and 3rd weeks after the 3rd epicutaneous induction. 1st challenge: - Applied to the left flank of test article and control treated animals: 6 and 3% - Applied to the right flank of the test article and control treated animals: saline. At the end of the exposure period (6 hours), test article and 2nd control was removed with saline solution. Skin reactions were recorded at 24, 48 and 72 hours after the challenge applications.
3. Evaluation criteria:	No visible change: 0 Slight localised redness 0.5 Slight redness 1 Moderate redness 2 Severe redness 3
4. Interpretation criteria:	Evidence of skin sensitization potential was evaluated against the following criteria: - Redness (score ≥ 1) in 15% of the test animals using the non-adjuvant test.
5. Statistics:	Not undertaken.

C. Methods:

1. Homogeneity and achieved concentration analysis of the dose:	Not undertaken.
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 24, 48 and 72 hours after the challenge applications.
3. Body weights:	Animals were weighed prior to study start, day 31 and 38.
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:

After assessment at 24, 48 and 72 hours, test sites administered spiroxamine at 25, 50 and 100% display erythema at all time points. At 12%, only a single animal exhibited mid slight localized erythema at 72 hours.

Table CP 7.1.6/01-1: Overview of Buehler skin sensitisation study in guinea pigs treated with Spiroxamine EC 500 (500 g/L): scores according to the Buehler grading for the preliminary range finder animals

0.5%			1%			3%			6%		
24 h	48 h	72 h	24 h	48 h	72 h	24 h	48 h	72 h	24 h	48 h	72 h
no. of animals with skin reddening/total no. of animals treated											
0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	1/5	1/5

12%			25%			50%			100		
24 h	48 h	72 h	24 h	48 h	72 h	24 h	48 h	72 h	24 h	48 h	72 h
no. of animals with skin reddening/total no. of animals treated											
3/5	4/5	4/5	5[a]/5	5[a]/5	5[a,b]/5	5[a]/5	5[a]/5	5[a,b]/5	5[a]/5	5[a]/5	5[a]/5

a treatment site squamous in places
b treatment site encrusted in places

C. Observations:

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

2. Mortality: No deaths were observed, with all animals surviving until the end of the observation period (day 31).

3. Skin reactions: After challenge no difference with regard to the incidence and intensity of skin reactions were seen between the test article treated animals and control group animals at both concentrations (3 and 6%) tested.

Total skin reactions at 24, 48 and 72 hours confirmed 0% of test article treated animals exhibiting erythema at both test sites.

The<15% animals exhibiting a skin erythema, Spiroxamine 500 g/L EC is considered a not a skin sensitiser.

Without a concurrent positive control or historical control data, the sensitivity and specificity of the test system was not demonstrated at the laboratory.

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 Buehler skin sensitisation study in guinea pigs treated with Spiroxamine EC 500 (500 g/L): body weight

Day	Control group	Spiroxamine 500 g/L EC	
		3%	6%
0	354 ±18	356 ±18	368 ±21
31	581 ±36	583 ±36	647 ±34
Net body weight gain (g)	227 ±28.8	227 ±33.0	280 ±22.4

E. Necropsy:

Not conducted

F. Deficiencies:

Although the study was conducted according to test guideline OECD 406 (1981), this test guideline has since been updated in the intervening period (1992). When assessed against current test guideline requirements the following deficiencies are 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 argument can be made that the without a concurrent positive control, or historical control data presented the sensitivity and specificity of the test system was not demonstrated at the laboratory. The data generated were sufficient to conclude that spiroxamine is a skin sensitiser, which is confirmed in an independent study employing a different model.
- In conclusion, the data generated under this study are considered supplementary with the skin sensitisation endpoint conclusively addressed with a guideline compliant local lymph node assay conducted in mice (refer to CP 7.1.6/02 [M-303647-01-1]).

Conclusions

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, Spiroxamine 500 g/L EC was confirmed to not be a skin sensitiser when examined in the guinea pig employing the Buhler methodology. These data however are considered supplementary as the sensitivity and specificity of the test system at the conducting laboratory had not been demonstrated.

Data Point:	KCP 7.1.6/02
Report Author:	[redacted]
Report Year:	2008
Report Title:	Spiroxamine EC 500 G (Project: Spiroxamine (KWG 4168)) - Local lymph node assay in mice (LLNA/IMDS)
Report No:	AT04681
Document No:	M-303647-01-1
Guideline(s) followed in study:	OECD 406; OECD 429; Guideline 2004/73/EC, Method B.6., B.42.; US-EPA 712-C-03-197, OPPTS 870.2600
Deviations from current test guideline:	Yes Although the study was conducted according to test guideline OECD 429 (2002), this test guideline has since been updated in the intervening period (2010). When assessed against current test guideline requirements the following deficiencies are noted: Radioactivity (incorporation of tritiated methyl thymidine) is typically used to measure lymph node cell proliferation. However, in the modified version of the test methodology reported in this study, a combination of absolute lymph node cell counts, lymph node weights and ear swelling were undertaken. This approach is different from the non-radioactive version of the test guideline, OECD 442A, which uses a bioluminescent method utilising the luciferase enzyme to catalyse the formation of light from ATP and luciferin.
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 modified local lymph node assay (LLNA-OECD 429, 2002) was conducted in Hsd Win mice in order to examine the delayed skin sensitisation potential of Spiroxamine 500 g/L EC. The modified methodology utilised did not involve assessing proliferation following the incorporation of tritiated

thymidine, but rather a combination of absolute lymph node cell counts, lymph node weights and ear swelling. Groups of mice (6/group) received the test article formulation at doses of 0, 2, 10, 50% applied to the auricles of each ear (25 µL) for 3 consecutive days.

The reliability of the assay was confirmed periodically by the conducting laboratory with the positive control, α-hexylcinnamaldehyde (HCA) applied as previously described at concentrations of 3, 10 and 30%.

On day 4 of the study ear weights of the sacrificed animals were measured using a punch to take a piece of ear with a diameter of 8 mm. Weights were determined from this ear punch. The left and right draining auricular lymph nodes from each mouse ear were excised, weighed and used for auricular lymph nodes weight and cell count analysis

A statistically significant increase ($p \leq 0.05$) in ear weight was observed in animals that received Spiroxamine 500 g/L EC at 10 and 50% on day 4, with relative increases of 16% and 60%, respectively.

A statistically significant increase ($p \leq 0.05$) in ear thickness was observed in animals that received Spiroxamine 500 g/L EC at 10 and 50% on day 4. The trigger level of ≥10% ear swelling for determining a positive effect was observed at a dose concentration of 50%, with relative increase of 37%, thus confirming that the highest concentration tested was irritant.

Relative auricular lymph node weights were statistically significant increased ($p \leq 0.05$) at a dose concentration of 50%. The stimulation index (SI) trigger level ≥1.4 for determining a positive effect was observed at a dose concentration of 50%, with an SI of 2.88.

Relative auricular lymph node cell counts were statistically significant increased ($p \leq 0.05$) at a dose concentration of 50%. The SI trigger level ≥1.4 for determining a positive effect was observed at a dose concentration of 50%, with an SI of 2.37. It is prudent to acknowledge that a dose concentration of 10%, the SI, whilst not statistically significant increased, was marginally below the trigger level at 1.37.

Collectively, the data generated do not fully follow either OECD 429 or OECD 442B test guideline, with the data only providing supplementary information. However, the active ingredient, spiroxamine is confirmed to be a skin sensitiser in studies using the Buehler method and the Maximization method (CA 5.2.6/01 [M-016682-01-1] and CA 5.2.6/02 [M-006309-01-1], respectively). Therefore, in accordance with Annex I for Regulation (EC) 1272/2008, as the generic concentration limit of the ingredient within the formulation exceeds the trigger level of 0.1%, Spiroxamine 500g/L EC is classified as Skin Sensitisation Category 1, H317 (may cause an allergic skin reaction).

Under the conditions of this study the test article, Spiroxamine 500 g/L EC was confirmed to be a skin sensitiser when examined in mice employing a non-test guideline modified LLNA methodology. Therefore, according to Annex I for Regulation (EC) 1272/2008, Spiroxamine 500g/L EC is classified as Skin Sensitisation Category 1, H317 (may cause an allergic skin reaction).

Materials and Methods

A. Materials:

1. Test Material:	Spiroxamine 500 g/L EC (alternative name: KWG 4168 500 EC; Spiroxamine EC 500 G)
Description:	Brown liquid
Lot/Batch No.:	PF90087683
Purity:	498 g spiroxamine/L (49.8% w/w)
CAS No.:	118134-30-8 (active ingredient)
Stability of test compound:	Confirmed stable for the duration of the study (expiry date: 31 January 2010)
2. Vehicle and/or positive control:	Pluronic PE 9200, 0.9% NaCl, 1% v/v/α-hexylcinnamaldehyde (HCA)

3. Test animals:

Species:	Mouse
Strain:	Hsd Win:NMRI
Age at dosing:	♀: 9 wks
Weight at dosing:	♀: 26-32g
Source:	[redacted]
Acclimation period:	At least 7 days
Diet:	PROVIMI KLIBA SA 3883 maintenance diet for rats and mice, *ad libitum*
Water:	Municipal water, *ad libitum*
Housing:	Housed 8 animals/cage during acclimatisation, singly housed during study phase

4. Environmental conditions:

Temperature:	22 ±2°C
Humidity:	55 ±15°C
Air changes:	*ca.* 10/h
Photoperiod:	12 hour light/dark

B. Study Design:

1. In life dates: 5 May 2008 to 8 May 2008 (experimental dates)

2. Test article formulation preparation: The test article formulations were prepared immediately prior to dosing in Pluronic PE 9200/0.9% NaCl solution at concentrations of 0 (vehicle control), 2, 10 and 50%.

3. LLNA assay: Mice (6/group) received the test article formulation at doses of 0, 2, 10, 50% applied to the auricles of each ear (25 µL) for 3 consecutive days.
The reliability of the assay was confirmed periodically by the conducting laboratory with the positive control, α-hexylcinnamaldehyde (HCA) applied as previously described at concentrations of 3, 10 and 30%.
Measurement of cell proliferation:
The auricular lymph nodes were disrupted and cell suspensions prepared. The cell suspension was dispensed into a 12-well flat bottom plate, the cell counts/mL were determined using a Multisizer 3®.
The ELNI was calculated separately based on lymph node cell count and lymph node weight:

$$SI = \frac{\text{Absolute cell count of the test article treated lymph nodes}}{\text{Absolute cell count of the vehicle control treated lymph nodes}}$$

$$SI = \frac{\text{Absolute weight of the test article treated lymph nodes}}{\text{Absolute weight of the vehicle control treated lymph nodes}}$$

4. Evaluation: For a test article to be considered as positive the following criteria were used:
- Group mean SI value of ≥ 1.4;
- Ear swelling ≥10% of the concurrent vehicle control

6. Statistics: Values from treated groups were compared with those from the concurrent vehicle control group by a one-way analysis of variance (ANOVA) when the variances were considered homogeneous according to a homogeneity testing like Cochran's test. Alternatively, if the variances were considered to be heterogeneous ($p<0.05$), a non-parametric Kruskal-Wallis test was used (Kruskal-Wallis ANOVA) at significance levels of 5%. Two sided multiple test procedures were done according to Dunnett or Bonferroni-Holm, respectively. Outlying values in the LN weights were eliminated at a probability level of 99% by Nalimov's method.

C. Methods:

1. Homogeneity and achieved concentration analysis of the dose:	Not undertaken.
2. Observations:	No observations recorded.
3. Body weights:	Animals were weighed prior to study start, end of day 1 and day 4.
4. Food consumption:	Not recorded.
5. Ear weight:	On day 4 of the study ear weights of the sacrificed animals were measured using a punch to take a piece of ear with a diameter of 8 mm, weights were determined from this ear punch.
5. Sacrifice and pathology:	All animals were killed at terminal sacrifice and the left and right draining auricular lymph nodes from each mouse ear were excised, weighed and used for auricular lymph nodes weight and cell count analysis (as detailed above).

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:	Not recorded.
2. Mortality:	No deaths occurred during the study.
3. Body weights:	No body weight effects were observed, with all animals gaining weight over the period.

C. Necropsy:

1. Ear weight:	A statistically significant increase ($p \leq 0.05$) in ear weight was observed in animals that received Spiroxamine 500 g/L EC at 10 and 50% on day 4, with relative increases of 16% and 60%, respectively.
2. Ear swelling:	A statistically significant increase ($p \leq 0.05$) in ear thickness was observed in animals that received Spiroxamine 500 g/L EC at 10 and 50% on day 4. The trigger level of ≥10% ear swelling for determining a positive effect was observed at a dose concentration of 50%, with relative increase of 37%, thus confirming that the highest concentration tested was irritant.
2. Auricular lymph nodes weight	Relative auricular lymph node weights were statistically significant increased ($p \leq 0.05$) at a dose concentration of 50%. The stimulation index (SI) trigger level ≥1.4 for determining a positive effect was observed at a dose concentration of 50%, with SI of 2.88.
3. Auricular lymph node cell count:	Relative auricular lymph node cell counts were statistically significant increased ($p \leq 0.05$) at a dose concentration of 50%. The SI trigger level ≥1.4 for determining a positive effect was observed at a dose concentration of 50%, with SI of 2.37. It is prudent to acknowledge that a dose concentration of 10%, the SI, whilst not statistically significant increased was marginally below the trigger level at 1.37.

Table CP 7.1.6/02-1: Overview of modified LLNA skin sensitisation study in mice treated with Spiroxamine EC 500 (500 g/L): body weight, LN and ear observations

Parameter	Day	Dose level (%)			
		0	2	10	50
Body wt (g)	0	28.8 ±1.83	29.8 ±1.83	28.8 ±1.17	28.2 ±1.47
	4	28.5 ±1.64	29.3 ±2.58	27.7 ±0.82	25.7 ±1.86
Relative LLN weight index		1.00	1.06	1.21	2.88*
LLN cell count (10^2cells/mL LLN suspension)		8270.92 ±929.89	9137.00 ±3124.23	11305.00 ±4311.83	22596.83 ±1347.60
LLN cell count index		1.00	1.10	1.37	2.37*
Ear swelling (10^{-2} mm ±%SD)	0	17.83 ±4.68	18.67 ±4.17	18.25 ±6.24	18.17 ±4.60
	4	17.92 ±5.03	18.58 ±5.36	19.58 ±3.95*	24.50 ±11.48*
Relative ear swelling index	4	1.00	1.04	1.09	1.37
Ear weight (mg/8 mm diameter punch ±%SD)		11.73 ±6.92	12.24 ±5.50	13.65 ±7.37	18.78 ±11.41*
Relative ear weight index		1.00	1.04	1.16	1.60

SD: standard deviation
LLN: local lymph node

D. Deficiencies:

When the study methodology is compared to current test guideline requirements (OECD 429, 2010) the following deficiencies are noted:

- Radioactivity (incorporation of tritiated methyl thymidine) is typically used to measure lymph node cell proliferation. However, in the modified version of the test methodology reported in this study, a combination of absolute lymph node cell counts, lymph node weights and ear swelling were undertaken. This approach is different from the non-radioactivity version of the test guideline, OECD 442A, which uses a bioluminescent method utilising the luciferase enzyme to catalyse the formation of light from ATP and luciferin.
- Collectively, the data generated do not fully follow either OECD 429 or OECD 442B test guideline, with the data only providing supplementary information. However, the active ingredient, spiroxamine is confirmed to be a skin sensitiser in studies using the Buehler method and the Maximization method (CA 5.2.6/01 [M-016682-01-1] and CA 5.2.6/02 [M-006309-01-1], respectively). Therefore, in accordance with Annex I for Regulation (EC) 1272/2008, as the generic concentration limit of the ingredient within the formulation exceeds the trigger level of 0.1%, Spiroxamine 500g/L EC is classified as Skin Sensitisation Category 1, H317 (may cause an allergic skin reaction).

However, the results produced under the modified LLNA methodology are deemed valid, with the data assessed collectively with available data on the active ingredient to conclude on skin sensitisation potential of the formulation, Spiroxamine 500 g/L EC.

Conclusions

<u>**Assessment and conclusion by applicant:**</u>
Assessment: This study is deemed acceptable and meets the requirements in 284/2013.
Conclusion: Under the conditions of this study the test article, Spiroxamine 500 g/L EC was confirmed to be a skin sensitiser when examined in mice employing a non-test guideline modified LLNA methodology. Therefore, according to Annex I for Regulation (EC) 1272/2008, Spiroxamine 500g/L EC is classified as Skin Sensitisation Category 1, H317 (may cause an allergic skin reaction).

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 or other components in Spiroxamine EC 500 that would require further investigations.

CP 7.1.8 Supplementary studies for combinations of plant protection products

No such studies are necessary since Spiroxamine EC 500 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 spiroxamine when used in the Spiroxamine EC 500 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 Spiroxamine EC 500 (500 g/L) for exposure assessment

Crop (field / indoor)	No. of applications (interval)	Application rate (kg a.s/ha)	Water volume L/ha	Application equipment
Grape [early/late application] (field) [BBCH 13-85 / BBCH 53-85]	1 – 2[a] (10 d interval)	Max rate/appli: 0.3	150[b] – 1000	Tractor-mounted conventional air blast 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 air blast sprayers for grapes 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.

The input parameters for the EFSA model calculations are shown in:

- Table CP 7.2-2: grape

The default body weight using the EFSA model is 60 kg.

Table CP 7.2-2 Input parameters for the EFSA model for the active substance spiroxamine when applied to grapes (field)

Substance name	Spiroxamine	
Product name	Spiroxamine 500 g/L EC	
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	Grapes	
Substance properties		
Formulation type	Soluble concentrates, emulsifiable concentrate, etc.	
Miniumum volume water for application (liquids)	150	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	µg/cm² of foliage/kg a.s. applied/ha
Dermal absorption of product	1.60%	
Dermal absorption of in-use dilution	32.00%	
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	Upward spraying	
Application equipment	Vehicle-mounted-Drift Reduction	
Buffer strip	10	m
Number of applications	2	
Interval between multiple applications	10	days
Season (upward spraying orchards only)	not relevant	

CP 7.2.1 Operator exposure

The application of Spiroxamine EC 500 (500 g/L) to the various crops 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 of 1.6% and 32% for the concentrate and spray dilutions of Spiroxamine EC 500 (500 g/L), respectively were used (see Section CP 7.3).

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

Model data	Level of PPE	Total absorbed dose (mg/kg bw/d)		%AOEL (0.015 mg/kg bw/d)	%AAOEL (0.061 mg/kg bw)	Reference
		Long term	Short term			
Tractor-mounted air blast sprayer application outdoors to grapes *Application rate: 0.6 L product/ha (0.3 kg spiroxamine/ha)*						
EFSA model • 10 ha/day[1] • 5 m buffer[1] • 60 kg[3]	No PPE[2]	0.2023	0.3896	1348.99	638.70	Table CP 7.2-2 (input parameter)
	Protective garment[4]	0.0604	0.2332	402.72	382.35	Table CP 7.2.1.1-3 (exposure estimate)
	PPE a-only[5]	0.0085	0.0401	56.46	65.78	Table CP 7.2.1.1-4 (exposure estimate)

1 Default value for high crops - tractor-mounted air blast sprayer
2 No PPE defined as operator not wearing a coverall or gloves
3 Default body weight for EFSA model
4 Protective garment defined as operator wearing a work wear clothing covering arms, body and legs
5 PPE in the form of gloves, hood and visor worn during application only

Conclusion

According to the EFSA (2015) model calculations it can be concluded that when operators are applying Spiroxamine EC 500 (500 g/L) outdoors to the representative crop using tractor-mounted air blast sprayer application without PPE, potential exposure is 1349% of the AOEL (0.015 mg/kg bw/day) for long term exposure, and 1768% of the AAOEL (0.061 mg/kg bw) for short term exposure. Work wear covering the arms, body and legs results in a marked reduction in systemic exposure, but PPE in the form of gloves, hood and visor during application is required to markedly reduce the systemic exposure below the AOEL and AAOEL for long and short term exposure, respectively.

Thus, Spiroxamine EC 500 (500 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. 1, H318; Skin Sensitisation, Cat. 1, H317) PPE in the form of gloves and hood to protect eyes and skin is recommended during mixing/loading and application.

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 Spiroxamine EC 500 (500 g/L) to grapes. 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
High outdoor crops (grapes)	10 ha/day (default for tractor-mounted air blast sprayer)	0.3 kg as/ha

Table CP 7.2.1.1-2 Penetration and absorption data

Category of absorption	Penetration/absorption rate	Reference
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)
Dermal absorption through exposure to the concentrate (mixing/loading)	1.6%	CP 7.3/01 M-398018-01-1
Dermal absorption through exposure to the spray dilution	32%	CP 7.3/03 M-761550-01-1

AOEL: 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%)

AAOEL: 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 AOEL of 0.015 mg/kg bw/day and AAOEL of 0.061 mg/kg bw, for long and short term system exposure, 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 Operator outdoor spray AOEM results for field application of Spiroxamine EC 500 (500 g/L) to grapes (0.3 kg a.s./ha) without PPE – tractor-mounted air blast sprayer application

Operator Model		Mixing, loading and application AOEM		
Potential exposure	Longer term systemic exposure (mg/kg bw/day)	0.2023	% of RVNAS	1348.99%
	Acute systemic exposure (mg/kg bw/day)	0.3896	% of RVAAS	638.70%
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)	0.0604	% of RVNAS	402.72%
	Acute systemic exposure (mg/kg bw/day)	0.2332	% of RVAAS	382.35%

Table CP 7.2.1.1-4 Operator outdoor spray AOEM results for field application of Spiroxamine EC 500 (500 g/L) to grapes (0.3 kg a.s./ha) with PPE (gloves, hood + visor) worn during application – tractor-mounted air blast sprayer application

Operator Model	Mixing, loading and application AOEM			
Potential exposure	Longer term systemic exposure (mg/kg bw/day)	0.2023	% of RVNAS	1348.99%
	Acute systemic exposure (mg/kg bw/day)	0.3896	% of RVAAS	638.70%
Mixing and Loading	Gloves = No	Clothing = Work wear - arms, body and legs covered	RPE = None	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)	0.0077	% of RVNAS	51.45%
	Acute systemic exposure (mg/kg bw/day)	0.0401	% of RVAAS	65.78%

Conclusion

According to the EFSA (2015) model calculations it can be concluded that when operators are applying Spiroxamine EC 500 (500 g/L) outdoors to the representative crop using tractor-mounted air blast sprayer application without PPE, potential exposure is 1349% of the AOEL (0.015 mg/kg bw/day) for long term exposure, and 638.70% of the AAOEL (0.016 mg/kg bw) for short term exposure. Work wear covering the arms, body and legs results in a marked reduction in systemic exposure, but PPE in the form of gloves, hood and visor during application is required to reduce the systemic exposure below the AOEL and AAOEL for long and short term exposure, respectively.

Thus, Spiroxamine EC 500 (500 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. 1, H318; Skin Sensitisation, Cat. 1, H317) PPE in the form of gloves and hood to protect eye and skin is recommended during mixing/loading and application.

CP 7.2.1.2 Measurement of operator exposure

Not required as assessments demonstrated safe use using the accepted models.

CP 7.2.2 Bystander and resident exposure

Bystander and resident exposures were conducted using the EFSA (2015) model.

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 7.2.2-1 and Table CP 7.2.2-2, respectively. 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. Drift reduction and increased 0 m buffer strip are included in the evaluation.

Table CP 7.2.2-1 Summary of estimations of bystander exposure in relation to the AAOEL using the EFSA model

Model data	Age group	Absorbed dose (mg/kg bw/d)				% AAOEL (0.061 mg/kg bw)	Reference
		Spray drift	Vapour	Surface deposits	Entry into treated crops		
Tractor-mounted air blast sprayer application outdoors to grapes *Application rate: 0.6 L product/ha (0.3 kg spiroxamine/ha)*							
EFSA model • 10 kg[1] • 60 kg[2]	Child	**0.1019**	0.0011	0.0006	0.0291	1.75 – **167.04%**	Table CP 7.2-1 (input parameter) Table CP 7.2.2.1-1 (exposure estimate)
	Adult	0.0565	0.0002	0.0003	0.0161	0.38 – 92.61%	

Absorbed dose values presented in **bold** exceed the assigned AOEL
1 Default child body weight
2 Default adult body weight

Table CP 7.2.2-2 Summary of estimations of resident exposure in relation to the AOEL using the EFSA model

Model data	Age group	Absorbed dose (mg/kg bw/d)					% AOEL (0.015 mg/kg bw/d)	Reference
		Spray drift	Vapour	Surface deposits	Entry into treated crops	All pathways (mean)		
Tractor-mounted air blast sprayer application outdoors to grapes *Application rate: 0.6 L product/ha (0.3 kg spiroxamine/ha)*								
Bystander								
EFSA model • 10 kg[1] • 60 kg[2]	Child	**0.0445**	0.0011	0.0003	**0.0291**	**0.0541**	**357.93%**	Table CP 7.2-1 (input parameter) Table CP 7.2.2.1-2 (exposure estimate)
	Adult	**0.0247**	0.0002	0.0001	**0.0161**	**0.0293**	**196.36%**	

Absorbed dose values presented in **bold** exceed the assigned AOEL
1 Default child body weight
2 Default adult body weight

Conclusion

According to the EFSA (2015) model, child bystander and adult and child resident exposures are significantly higher than the AAOEL (0.061 mg/kg bw) and AOEL (0.015 mg/kg bw/day), respectively. Consequently, whilst it is acknowledge that at present an acceptable risk for both bystanders and residents cannot be demonstrated following application of Spiroxamine EC 500 (500 g/L) to outdoor crops, an impending study to investigate systemic exposure to this population cohort is to be undertaken.

CP 7.2.2.1 Estimation of bystander and resident exposure

Bystanders and residents are defined as persons who are not intentionally involved in the application or application related activities. They might be temporarily located in the vicinity of an application (in the following called "bystander") or working or living in the vicinity of the application (in the following called "resident").

The exposure estimates for bystanders and residents are calculated using the EFSA (2015) model. All assumptions made in the model are explained in the EFSA guidance and are not detailed here.

Four pathways of exposure are considered:

- spray drift (at the time of application)
- vapour (may occur after the PPP has been applied)
- surface deposits
- entry into treated crops.

Summing all the exposure pathways, each one being conservative would result in an overly unrealistic result for bystanders, therefore each pathway is presented separately.

It is conservatively assumed for a Tier 1 assessment that total systemic exposure is the sum of the mean exposures from each pathway, with residential exposures based on the 75th percentile estimates. The worst-case dermal absorption value of 32% for the spray dilution of Spiroxamine EC 500 (500 g/L) was used to derive the systemic dermal exposure (see Section CP 7.3). The algorithms used to estimate bystander and resident exposures are explained in the EFSA (2015) guidance.

The input parameters are shown in Table CP 7.2-2. The adult and child (less than 3 years old) body weights are 60 kg and 10 kg, respectively. Oral absorption (applicable for children) is deemed to be 61%.

The estimated exposure were compared with the AAOEL of 0.061 mg/kg bw for bystander exposure and the AOEL of 0.015 mg/kg bw/day for resident exposure.

Table CP 7.2.2.1-1: Bystander exposure results for field application Spiroxamine EC 500 (500 g/L) to grapes (0.3 kg a.s./ha) – tractor-mounted air blast sprayer application using the EFSA model

Bystander - child	Spray drift (95th percentile) (mg/kg bw/day)	0.1019	% of RVAAS	167.04%
	Vapour (95th percentile) (mg/kg bw/day)	0.0011	% of RVAAS	1.75%
	Surface deposits (95th percentile) (mg/kg bw/day)	0.0006	% of RVAAS	0.99%
	Entry into treated crops (95th percentile) (mg/kg bw/day)	0.0291	% of RVAAS	47.64%
Bystander - adult	Spray drift (95th percentile) (mg/kg bw/day)	0.0565	% of RVAAS	92.61%
	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	0.42%
	Entry into treated crops (95th percentile) (mg/kg bw/day)	0.0161	% of RVAAS	26.46%

Table CP 7.2.2.1-2: Resident exposure results for field application Spiroxamine EC 500 (500 g/L) to grapes (0.3 kg a.s./ha) – tractor-mounted air blast sprayer application using the EFSA model

Resident - child	Spray drift (75th percentile) (mg/kg bw/day)	0.0445	% of RVNAS	296.56%
	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	1.68%
	Entry into treated crops (75th percentile) (mg/kg bw/day)	0.0291	% of RVNAS	193.72%
	All pathways (mean) mg/kg bw/day	0.0537	% of RVNAS	357.93%
Resident - adult	Spray drift (75th percentile) (mg/kg bw/day)	0.0247	% of RVNAS	164.38%
	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	0.71%
	Entry into treated crops (75th percentile) (mg/kg bw/day)	0.0161	% of RVNAS	107.62%
	All pathways (mean) mg/kg bw/day	0.0293	% of RVNAS	195.36%

Conclusion

According to the EFSA (2015) model, child bystander and adult and child resident exposures are significantly higher than the AAOEL (0.061 mg/kg bw) and AOEL (0.015 mg/kg bw/day), respectively.

Consequently, whilst it is acknowledge that at present an acceptable risk for both bystanders and residents cannot be demonstrated following application of Spiroxamine EC 500 (500 g/L) to outdoor crops, an impending study to investigate systemic exposure to this population cohort is to be undertaken.

CP 7.2.2.2 Measurement of bystander and resident exposure

Since the exposure estimate carried out indicated that the AAOEL and AOEL for bystanders and residents, respectively will be exceeded under practical conditions of use, a study to provide a measurement of bystander/resident exposure is to be undertaken. The intention was that this work would have been conducted end of Q3, 2020 with discussion with the laboratory undertaken January 2020. However due to the global SARS CoV-2 (Severe acute respiratory syndrome coronavirus 2) pandemic, declared by WHO (World Health Organisation) as PHEIC (Public Health Emergency of International Concern) proposed start dates have had to be delayed by a complete season, with the study due to start June 2021 and the draft report available January 2022. This was incorporated into the protocol prior to finalization. A statement from the contract organization can be obtained, if requested.

Dossier node	Draft title	Study ID	Planned submission
CP 7.2.2.2/01	determination of bystander-resident dermal and inhalation exposure to spiroxamine during foliar application wo wine grapes in norther and southern Europe	S20-03916	Final: 1st Quarter 2022

CP 7.2.3 Worker exposure

Worker exposures from re-entry to treated crops were estimated using the EFSA (2015) model. As there are manual harvesting activities associated with the representative crops, consequently re-entry activities involving contact with treated crops include crop inspection (deemed to last no longer than 2 hours) or harvesting activities (8 hours).

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

Model data	Level of PPE	Total absorbed dose (mg/kg bw/d)	% AOEL (0.015 mg/kg bw/d)	Reference
EFSA model • Grapes • Outdoor • 0.3 kg a.s/ha • 2 application	Potential exposure[1]	2.0663	13775.62	Table CP 7.2-2 (input parameter) Table CP 7.2.3.1-2 (exposure estimate)
	Work wear[2]	0.6957	4637.79	
	Work wear + gloves	---[3]	---[3]	

1 No work wear
2 Clothing covering arms, body, legs
3 Data not available in the EFSA model to estimate systemic exposure when PPE are worn

Conclusion

According to the EFSA (2015) model, worker exposures are significantly higher than the AOEL (0.015 mg/kg bw/day). Consequently, whilst it is acknowledge that at present an acceptable risk for workers cannot be demonstrated following application of Spiroxamine EC 500 (500 g/L) to outdoor

crops, an impending study to investigate systemic exposure to this population cohort is to be undertaken. It is prudent to acknowledge due to the global SARS CoV-2 pandemic, this work has had to be delayed until the 2021 season.

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/cm². 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 Spiroxamine EC 500 (500 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 (cm²/h)		
	Total potential exp.	Arms, body, legs covered	Hands, arms, body, legs covered[1]
Grapes	30000	10100	-

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

Table CP 7.2.3.1-2 Worker exposure (long term exposure) results for field application of Spiroxamine EC 500 (500 g/L) to grapes (0.3 kg a.s./ha)

Worker - Hand harvesting	Potential exposure (mg/kg bw/day)	2.0663	% of RVNAS	13775.62%
	Working clothing (mg/kg bw/day)	0.6957	% of RVNAS	4637.79%
	Working clothing and gloves (mg/kg bw/day)		% of RVNAS	

Conclusion

According to the EFSA (2015) model, worker exposures are significantly higher than the AOEL (0.015 mg/kg bw/day). Consequently, whilst it is acknowledge that at present an acceptable risk for workers cannot be demonstrated following application of Spiroxamine EC 500 (500 g/L) to outdoor crops, an impending study to investigate systemic exposure to this population cohort is to be undertaken. It is prudent to acknowledge due to the global SARS CoV-2 pandemic, this work has had to be delayed until the 2021 season.

CP 7.2.3.2 Measurement of worker exposure

Since the exposure estimate carried out indicated that the AAOEL and AOEL for bystanders and residents, respectively will be exceeded under practical conditions of use, a study to provide a measure of bystander/resident exposure is to be undertaken. The intention was that this work would have been

conducted end of Q3, 2020 with discussion with the laboratory undertaken January 2020. However due to the global SARS CoV-2 (Severe acute respiratory syndrome coronavirus 2) pandemic, declared by WHO (World Health Organisation) as PHEIC (Public Health Emergency of International Concern) proposed start dates have had to be delayed by a complete season, with the study due to start June 2021 and the draft report available January 2022. This was incorporated into the protocol prior to finalization. A statement from the contract organization can be obtained, if requested.

Dossier node	Draft title	Study ID	Planned submission
CP 7.2.3.2/01	Spiroxamine EC 500 Determination of worker re-entry exposure to spiroxamine during shoot lifting grape in northern and southern Europe	S20-03917	Final: 1st Quarter 2022

CP 7.3 Dermal absorption

An existing *in vitro* dermal absorption study conducted in human skin, and interpreted inaccordance with the current EFSA dermal absorption guidance concludes dermal absorption for the active ingredient, spiroxamine in the Spiroxamine EC 500 formulation to be 1.6%. A new *in vitro* dermal absorption study conducted in the same skin type, at a spray dilution representative of the GAP confirmed that the dermal absorption of a spray dilution equivalent to 0.3 g/L (1:1667 dilution) following an 8 hour exposure is 32%. These values were used for the non-dietary risk assessment. Study M-398018-01-1 supersedes M-304386-01-1 (refer to baseline dossier) due to low recovery values obtained.

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

Endpoint	Dermal absorption values		Reference
Dermal penetration	Concentrate (500 g/L)	1.6%	CP 7.3/01 M-398018-01-1
	Spray dilution (0.3 g/L [1:1667 dilution])	32%	CP 7.3/03 M-761550-01-1

In vitro dermal absorption in human skin

Data Point:	KCP 7.3/01
Report Author:	[redacted]
Report Year:	2010
Report Title:	Impulse EC 500: [14C]-spiroxamine - Comparative in vitro dermal absorption study using human and rat skin
Report No:	SA 10186
Document No:	M-398018-01-1
Guideline(s) followed in study:	O.E.C.D. Guideline for the testing of Chemicals Skin Absorption In Vitro Method Guideline 428 (April 2004); O.E.C.D. Environmental Health and Safety Publication 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, (March 2004)
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 dermal absorption of spiroxamine from an emulsifiable concentrate (EC) formulation was studied using human and rat skin *in vitro*. Two concentrations were tested: a concentrate formulation of 500 g/L (neat formulation) and an in-use spray dilution of 0.75 g/L (diluted formulation). Although the study was undertaken on both human and rat skin, this summary focuses primarily on the human *in vitro* element of the study as these values are used for risk assessment.

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

The skin samples from at least four different donors were mounted into flow-through cells and the diffusion cell placed in water bath maintained at temperature of 32 ±2°C. The absorption process was followed by taking samples of the receptor fluid, Eagle's medium supplemented with 5% bovine serum albumin, 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, a solubility assessment and dose homogeneity were carried out. The barrier integrity was also assessed *via* trans-epidermal water loss (TEWL) measurement of the skin samples.

The mass balance for [^{14}C]-Spiroxamine in the formulation concentrate and spray dilution were 97.53% and 106.70%, respectively.

The study demonstrated that the amount of spiroxamine absorbed through human split-thickness skin over 24 h for the formulation concentrate (500 g/L) and spray dilution (0.75 g/L) was 0.63 ±0.47% and 18.21 ±2.31%, respectively, as measured in the skin, receptor fluid and receptor chamber. Using the current EFSA Guidance on Dermal Absorption 2017, 15(6): 4873, the estimate to be used for risk assessment is 1.6% and 23% for the formulation concentrate and spray dilution, respectively.

Table CP 7.3/01-1: Spiroxamine 500 g/L EC: summary of the mean dermal absorption results[1]

Test Preparation:	**Test preperation 1**		**Test preperation 2**	
Target concentration (g/L)	500		0.75	
Actual dose (g/L)	462		0.68	
Number of replicates	5		4	
	Recovery [%]			
	Mean	S.D	Mean	S.D
Dislodgeable dose				
Skin washing after 8 h (filter + swab)	96.20	3.58	75.26	9.36
Skin washing after 24 h (filter + swab)	0.21	0.26	5.86	5.53
Donor chamber wash	0.02	0.03	0.75	0.77
Dose associated to skin				
Tape strips: strips 1 + 2	0.54	0.18	3.46	3.77
Tape strips: strips 3+	0.23	0.17	3.16	3.06
Surrouding skin + swabs	0.03	0.05	0.05	0.03
Absorbed dose				
Skin	0.38	0.37	3.81	2.50
Receptor fluid	0.22	0.10	14.22	2.59
Receptor chamber wash	0.00	0.00	0.56	N.D
Total recovery[1]	97.57	3.03	106.69	1.26
Absorption essentially complete at end of study (>75% absorption within half the study duration) [%Absorption at $t_{0.5}$]	No [37.0%]		No [63%]	
Absorption estimate normalised[2]	No		Yes	
If no: Absorption estimates = absorbed dose + tape strips 3+)[3]	0.86	0.60	21.37	1.28
If yes: Absorption estimates = absorbed dose	Not applicable		Not applicable	
Relevant absorption estimate[4]	1.58		23.42	
Absorption estimates used for risk assessment[5]	**1.6**		**23**	

[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 n-th 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 + 1.6×SD (n=4), (mean + 1.2×SD (n=5)), based on Table 1 from the EFSA Guidance on dermal absorption, 2017. Based on the addition of tape strip 3+, surrounding skin + swabs and absorbed dose.

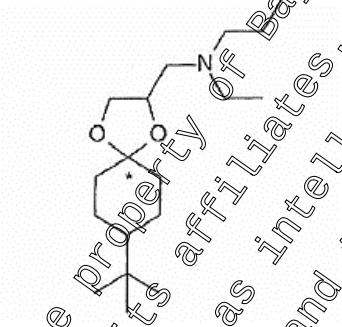
[5] Relevant absorption estimate was rounded to the required number of significant figures.

N.D not determined, based on only one value

Materials and methods

A. Materials:

1. Test Material (non-radiolabelled):	Spiroxamine
CAS number	118134-30-8
Description	Yellow liquid
Lot/Batch No.:	M28197
Purity:	97.4%
Stability of test compound:	Confirmed stable for the duration of the study (Expiry date: 17 October 2011)
2. Test Material (radiolabelled):	[cyclohexyl-1-^{14}C]-Spiroxamine



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

Lot/Batch No.:	SYP13550
Specific activity:	3.60 MBq/mg
Radiochemical purity:	97%
3. Blank formulation	Not applicable – specific radiolabel formulations were prepared by Bayer CropScience AG, Development
Lot/Batch No.:	Not applicable
Storage conditions	Not applicable
Nominal specific gravity / density	Not applicable
4. Test skin:	
Species:	Human
Sex:	♂
Age:	Not detailed
Site:	Abdomen
5. Preparation of dosing solutions	Formulations containing [^{14}C]-Spiroxamine were prepared at Bayer CropScience AG, Development. The neat formulation contained a nominal concentration of 500 mg spiroxamine/mL and had a radiochemical purity > 95%. The spray dilution contained a nominal concentration of 0.75 mg spiroxamine/mL and had a radiochemical purity of 95%.

B. Study Design and Methods:

1. In life dates:	23 August 2010 to 24 September 2010 (experimental dates)
2. Skin preparation:	Dermatomed human skin were obtained from a tissue bank. The samples had a thickness ranging between 446 and 565 µm Prior to dose application, skin integrity was assessed by measuring the trans-epidermal water loss (TEWL) from the *stratum corneum*. An evaporimeter probe was placed securely on the top of the donor chamber and the amount of water diffusing through the skin was measured. Any sample with a TEWL of

>15 g/hm^2 was considered potentially damaged and not used. Replacement fragments were also tested prior to their use.

3. Solubility of Spiroxamine in the receptor fluid:

The solubility in receptor fluid was investigated by mixing a volume of [^{14}C]-Spiroxamine with non-radiolabelled test substance, equating to the maximum dose to be applied to the cell. This was dissolved in approximately 3 mL (total cell volume) of the receptor fluid. This process was intended to simulate the maximum and instantaneous absorption of the applied dose. The samples were left for 24 hours at approximately 32°C. Thereafter, the samples were centrifuged (3000 rpm) for 10 minutes. Three aliquots were analysed by liquid scintillation counting (LSC). If the achieved concentrations were at least ten times lower than the determined solubility concentration then the solubility in the receptor fluid was deemed to be sufficient to reduce any risk of back diffusion.

4. Treatment:

A flow-through diffusion cell system was used for this study. The skin exposure area was 1 cm^2. The receptor fluid was Eagle's medium supplemented with 5% bovine serum albumin and gentamycin (50 mg/L) at a pH of 7.4. The receptor fluid was pumped through at a rate of 1.5 mL/h and stirred by magnetic bar. These cells were positioned in a manifold heated via a circulating water bath to maintain a skin surface temperature of 32 ±2°C. A single dose of 10 µL/cm^2 of the test preparation was applied evenly over the surface of 6 skin membranes. A filter containing activated charcoal was placed on the top of the diffusion cell to trap any potential spiroxamine that would evaporate. The dose preparations were assayed by LSC, using dose checks taken before, during and after the dosing process.

5. Sampling:

Absorption of [^{14}C]-Spiroxamine from the test preparation was assessed by collecting fractions of the receptor fluid at hourly intervals for 24 hours. Receptor fluid samples were weighed at 2, 12 and 22 hours to ensure the correct flow rate was maintained. The exposure period was terminated at 8 h post dose. The carbon filter was removed and retained for analysis. The skin was swabbed with 1% v/v Tween 80 in phosphate buffer saline (PBS) using natural sponge swabs, until no radioactivity was detected with a Geiger counter. A new carbon filter was placed on top of the diffusion cell when the process was complete.

24 hour after application, the filter was removed and retained for analysis. The treated skin and the skin adjacent to the treatment site (surrounding swabs) were swabbed. The *stratum corneum* was removed via the application of Monaderm adhesive tape. The tape was applied for 5 seconds and removed against the direction of hair growth, the process was repeated until the 'shiny'

appearance of the epidermis was evident. The skin surrounding the application site (surrounding skin) was separated from the treated skin, both elements of skin were retained for analysis.

6. Radioassay:

All samples prepared in scintillation fluid were subjected to LSC. 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 1900-TR scintillation counter. Samples were counted for 10 minutes or for 2 sigma %.

Receptor fluid: Scintillation fluid was directly added to each receptor fluid sample and to the receptor chamber/outlet tubing contents and analyzed by LSC.

Skin swabs: Swabs were solubilized using Soluene and analysed by LSC.

Tape-strips: individual samples were solubilized using tetrahydrofuran, prior to the addition of scintillation fluid for LSC analysis.

Charcoal filter: Filters were combusted separately, the radioactivity trapped before LSC measurement.

Skin membranes: Each membrane was separately solubilized using Soluene and scintillation fluid added for LSC analysis.

Diffusion cell components: All components were separately soaked mixture of acetonitrile/distilled water (50:50 v/v) for 12 hours. Following this were assessed with a Geiger counter. The samples were weighed and duplicate aliquots analysed by LSC.

7.Data interpretation: *Calculation of dermal absorption parameters:* Dislodgeable dose (skin wash 8 & 24 h + filters 8 & 24 h + donor wash), unabsorbed dose (total dislodgeable dose + *stratum corneum* + surrounding skin & swabs), absorbed dose (cumulative receptor fluid + residual 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.

Results

A. Dermal absorption:

1. Solubility of the test item in receptor fluid: The solubility of spiroxamine in the receptor fluid indicated that a concentration of 100 µg/mL of receptor fluid. The maximal concentration in the receptor fluid (per hour) during the study was 5.94 µg/mL. The achieved solubility concentration was ≤16.84 fold greater than the receptor fluid value.

2. Dose homogeneity: Based on five aliquots, the dose formulations had a coefficient of variation between samples ranged between 3.4 and 3.6% for all formulations tested (concentrate and spray dilution). Therefore, the dose formulation were considered homogenous.

3. Skin integrity test: The integrity of the reported skin samples was within the acceptability criteria (absorption (≤15 g/hm^2). All data is presented in full in the report.

4. Neat formulation (nominally 500 g/L): Six samples of human split-thickness skin membranes obtained from 6 different donors were dosed topically with [^{14}C]-Spiroxamine neat formulation (500 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 H01, which had a mass balance <90% of the applied dose. Therefore, this cell was rejected from further analysis. The following results are provided as mean values (n = 5). Following the wash at 8 h, 96.20% of the applied dose of [^{14}C]-Spiroxamine was washed off. At 24 h post dose, a further 0.21% was removed during the wash. A proportion of the dose applied was recovered from the donor chamber (0.02%), skin (0.38%) and receptor chamber wash (0.0%). The mean total recovery was 97.57% of the applied dose.

5. Spray dilution (nominally 0.75 g/L): Six samples of human split-thickness skin membranes obtained from 6 different donors were dosed topically with [^{14}C]-Spiroxamine spray dilution (0.75 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 H07 and H012, which had a mass balance >110% of the applied dose. Therefore, these cells were rejected from further analysis. The following results are provided as mean values (n = 4). Following the wash at 8 h, 75.26% of the applied dose of [^{14}C]-Spiroxamine was washed off. At 24 h post dose, a further 5.86% was removed during the wash. A proportion of the dose applied was recovered from the donor chamber (0.75%), skin (3.81%) and receptor chamber wash (0.56%). The mean total recovery was 106.69% 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 spiroxamine absorbed through human split-thickness skin over 24 h for the formulation concentrate (500 g/L) and spray dilution (0.75 g/L) was

0.63 ±0.47% and 18.21 ±2.31%, respectively, as measured in the skin, receptor fluid and receptor chamber. Using the current EFSA Guidance on Dermal Absorption 2017, 15(6): 4873, the estimate to be used for risk assessment is 1.6% and 23% for the formulation concentrate and spray dilution, respectively

Data Point:	KCP 7.3/03
Report Author:	[redacted]; [redacted]
Report Year:	2020
Report Title:	Spiroxamine EC 500: The In Vitro percutaneous absorption of radiolabelled spiroxamine in a single in-use dilution through Human split-thickness skin
Report No:	786255
Document No:	M-761550-01-1
Guideline(s) followed in study:	OECD 428: (2004); OECD No. 28 (2004); EFSA Journal 15(6): 4873), (2017)
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*. One concentration of in-use spray dilution was tested, 0.3 g/L.

The dose was applied at 10 µL/cm² 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 cells 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 7.43 ± 0.02, 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 this dilution was 92.79%. Therefore, the data absorption from all cells was normalised to 100%, as the mass balance was not consistently >95%.

The study demonstrated that the amount of spiroxamine absorbed through split-thickness over 24 h from 0.3 g/L was 22.47 ± 7.26%, 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 32% for 0.3 g/L.

Table CP 7.3/03-1:
Spiroxamine 500 g/L EC: summary of the mean dermal absorption results[1]

Test Preparation:	**Test preparation 1**	
Target concentration (g/L)	0.3	
Actual dose (g/L)	0.294	
Number of replicates	12	
	Recovery [%]	
	Mean	S.D.
Dislodgeable dose		
Skin washing after 8 h	58.55	8.52
Skin washing after 24 h	8.29	4.61
Donor chamber wash	0.62	0.41
Dose associated to skin		
Tape strips: strips 1 + 2	0.66	0.80
Tape strips: strips 3 - 20	2.16	2.71
Unexposed skin	0.04	0.02
Absorbed dose		
Exposed skin	2.33	1.07
Receptor fluid	19.38	6.03
Receptor chamber wash	0.76	0.32
Total recovery[1]	92.79	3.54
Absorption essentially complete at end of study (>75% absorption within half the study duration) [%Absorption at $t_{0.5}$]	No [59%]	
Absorption estimate normalised[2]	Yes	
If no: Absorption estimates = absorbed dose + tape strips 3-20)[3]	26.37	8.71
If yes: Absorption estimates = absorbed dose	Not applicable	
Relevant absorption estimate[4]	31.95	
Absorption estimates used for risk assessment[5]	**32**	

[2] 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.

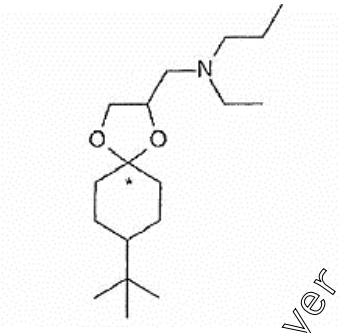
Materials and methods

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-1-14C]-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 EC500
Lot/Batch No.:	2020-003400
Storage conditions	Ambient/Protected from light
Nominal specific gravity / density	Not applicable
4. Test skin:	
Species:	Human
Sex:	3 Female, 1 Male
Age:	25 - 56 yrs
Site:	Abdomen

5. Preparation of dosing solutions

Test preparation 1: [^{14}C]-Spiroxamine stock solution (39.5 µL) was transferred to a glass vial and solvent was removed. Ultrapure water (475.14 mg) was added to a vial in aliquots already containing blank formulation (25.41 mg) and vortexed after each addition. This mixture (6.06 mg) was then added to the vial of [^{14}C]-Spiroxamine and 500 µL of ultrapure water was added. The contents were mixed on a magnetic stirring plate. The concentration of spiroxamine in the test preparation was too high. Therefore, the sample was further diluted, a 400 µL aliquot was placed into a separate vial and 230 µL of ultrapure water added. The radioactivity was quantified by liquid scintillation counting. The concentration of [^{14}C]-Spiroxamine by radioactivity was 0.294 g/L.

B. Study Design and Methods:

1. In life dates: 03 June 2020 to 30 June 2020 (experimental dates)

2. Skin preparation: Samples of full-thickness human skin (abdomen) were obtained from three female and one male donor aged 25 to 56 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 (20ºC). Theoretically, if 70% of spiroxamine was absorbed, this would result in a test item concentration in the receptor fluid of 2.7 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]-Spiroxamine 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 spiroxamine in the receptor fluid indicated that 92% 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 0.3 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 (0.3 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 7, which had a mass balance of 85.43% of the applied dose. However, this cell was not excluded, as the absorbed dose for Cell 7 was similar to the other cells from the same donor (1237). The absorption values from donor 1237 were lower in comparison to the other donors used. Therefore, it can be assumed that the lower absorption is attributable to the donor, and that the missing material for Cell 7 is attributable to the unabsorbed dose (e.g. dislodged dose). The mean mass balance was <95% of the applied dose. Therefore, all cells were normalised to 100%, as the mass balance was not consistently >95%.

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

Following the wash at 8 h, 58.55% of the applied dose of [^{14}C]-Spiroxamine was washed off. At 24 h post dose, a further 8.29% was removed during the wash. A proportion of the dose applied was recovered from the donor chamber (0.62%), exposed skin (2.33%) and receptor chamber wash (0.76%). The mean total recovery was 92.79% 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 spiroxamine absorbed through split-thickness over 24 h from 0.3 g/L was 22.47 ± 7.26%, 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 32% for 0.3 g/L.

CP 7.4 Available toxicological data relating to co-formulants

CONFIDENTIAL information – data provided separately (Document PCP).