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24-Epibrassinolide

Volume 3 – B.6 (PPP) – Sunergist

Rapporteur Member State : Austria

Version History

When	What
2018/05	Initial DAR

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B.6. TOXICOLOGY AND METABOLISM DATA AND ASSESSMENT OF RISKS FOR HUMANS

B.6.1. ACUTE TOXICITY OF PLANT PROTECTION PRODUCT

Results of acute toxicity studies performed with the formulation are summarized in the following table. No classification is proposed for the formulation.

Table 6.1-1: Summary of Available Guideline-Compliant Studies on Acute Toxicity with the Formulation

Parameter	Species	Result	Classification/ remarks	Reference
Acute oral toxicity	Wistar Rat	LD_{50} >5000 mg/kg bw (female)	Not required	[redacted] (2017)
Acute dermal toxicity	Wistar Rat	LD_{50}>2000 mg/kg	Not required	[redacted] (2017)
Acute inhalation (4 hours)	Not available	-	Study with active ingredient is available	-
Skin irritation	Young Adult New Zealand White Rabbits	Not skin irritant	Not required	[redacted] (2017)
Eye irritation	Young Adult New Zealand White Rabbits	Not eye irritant	Not required	[redacted] (2017)
Skin sensitisation (Magnusson and Kligman Method)	Albino Dunkin Hartley Guinea Pig	Non-sensitising	Not required	[redacted] (2017)

B.6.1.1. Oral

Annex point	KCP 7.1.1/01
Reference:	ACUTE ORAL TOXICITY STUDY IN RATS WITH SUNERGIST (EPIBRASSINOLIDE 0.01% SOLUBLE LIQUID)
Author(s), year:	[redacted] (2017)
Report/Doc. number::	6126 (526-001)
Guideline(s):	OECD No. 423 (2001), OPPTS 870.1000, OPPTS 870.1100
GLP:	Yes
Deviations from OECD 423 (2002):	none
Acceptability:	Yes

Executive summary

The study was performed to assess the oral toxicological potential of the test item Sunergist (Epibrassinolide 0.01% Soluble Liquid) in an *in vivo* test using female Wistar Rats. The test item was administered by oral gavage at a single dose of 5000 mg/kg body weight to three female animals. The test item was formulated in vehicle (Distilled water) at a concentration of 500 mg/mL. The dose volume was 10 mL/kg body weight. No clinical signs were observed and no substance related effects could be reported. No abnormalities were observed in any of the treated animals during necropsy at terminal sacrifice.

Based on the results, the median lethal dose of Sunergist (Epibrassinolide 0.01% Soluble Liquid) after single oral administration to female rats, observed over a period of 14 days is:

$LD_{50} \geq 5000$ mg/kg body weight

I. MATERIALS AND METHODS

A. Test material and vehicle

1 Test materials

Test substance	Sunergist (Epibrassinolide 0.01% SL)
CAS no.	78821-43-9
Lot/batch	002-20150506
Formulation Type	Epibrassinolide 0.01%; Surfactants and Solvents: 99.99%
Concentration	0.01 %
Expiry date	May 05, 2017
Storage conditions:	Room Temperature (20 °C to 30 °C)
Vehicle	Distilled water

2 Test animals

Species	Rat
Strain	Female Wistar Rats (*Rattus norvegicus)*
Age	9-11 weeks
Bodyweight at dosing	190.2 - 195.9 g
Source	[redacted]
Acclimation period	6 days
Diet	*Ad libitum* Teklad Certified Global 14% Protein Rodent Maintenance Diet (Batch No.2014C-111215MA) from ENVIGO
Water	*Ad libitum* tap water
Housing	Individually
Temperature	20.8 - 23.0 °C
Humidity	55 – 65 %
Ventilation	≥ 10 times/hour
Photoperiod	12 hours light/ 12 hours dark

B. Study design and method

Test substance preparation and administration

The test item Sunergist (Epibrassinolide 0.01% Soluble Liquid) was administered by oral gavage at a single dose of 5000 mg/kg body weight to three female animals. The test item was formulated in vehicle (Distilled water) at a concentration of 500 mg/mL. The dose volume was 10 mL/kg body weight. The administration volume was 10 mL/kg body weight. The animals were dosed using 15 G oral plastic feeding tubes.

Main test

One female rat received a single dose of the test item by oral gavage administration at 5000 mg/kg body weight. No mortality was observed in the single dosed animal and two female rats were subsequently treated at the same dose of 5000 mg/kg body weight. All animals survived throughout the experimental period, no further testing was carried out.

Mortality and clinical observations

The animals were observed twice daily during the acclimatization period and mortality/viability and clinical signs were recorded. All animals were observed for clinical signs during first 30 minutes and at approximately 1, 2, 3 and 4 hours post test item administration on test day 0 and once daily during test days 1-14. Mortality/viability was recorded during first 30 minutes and at approximately 1, 2, 3 and 4 hours post administration on test day 0 (in common with the clinical signs) and twice daily during days 1-14 (once on day of sacrifice).

Bodyweight

Body weights were recorded on test day 0 (prior to administration), test days 7 and 14.

Necropsy

All treated animals were sacrificed at the end of the observation period by carbon dioxide asphyxiation in euthanasia chamber and discarded after the gross/macroscopic pathological changes were observed and recorded. No organ and tissues were retained.

C Statistics

Data were not analysed statistically.

II. RESULTS AND DISCUSSION

Mortality and clinical signs

No mortality was observed in any of the animals treated at 5000 mg/kg body weight.

All the animals treated at the dose of 5000 mg/kg body weight did not display any clinical signs at approximately 30 minutes, at 1, 2, 3 and 4 hours on day 0 post test item administration until the last day of observation period (day 14).

Table 6.1.1-1: Acute oral toxicity: clinical data

Dose	Animal Number	Sex	Test days																	
			0*					1	2	3	4	5	6	7	8	9	10	11	12	13	14
			0.5	1	2	3	4														
5000 mg/kg body weight	01	F	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
	02	F	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
	03	F	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1

Key: mg/kg = milligram/kilogram, F= Female, 1= Normal,
No clinical signs were evident in any animal during the acclimatization period.
* Examinations were performed within the first 0.5 hours and at approximately 1, 2, 3 and 4 hours post treatment on test day 0.

Bodyweights
All the animals had gained body weight by day 7 and 14 as compared to day 0.

Table 6.1.1-2: Acute oral toxicity: bodyweights

Dose	Animal Number	Sex	Test day 0 (pre-treatment) (g)	Test day 7 (g)	Test day 14 (g)	Body Weight Gain (%) (Test day 0-7)	Body Weight Gain (%) (Test day 0-14)
5000 mg/kg body weight	01	Female	190.2	205.4	219.8	8.0	15.6
	02	Female	195.9	211.3	226.2	7.9	15.5
	03	Female	192.3	200.8	223.0	4.4	16.0

Key: mg/kg = milligram/kilogram, g = gram

Necropsy
No abnormalities were observed in any of the treated animals on necropsy at terminal sacrifice.

Table 6.1.1-3: Macroscopic findings

Dose	Animal Number	Sex	Mode of death	Macroscopic findings
5000 mg/kg body weight	01	Female	Terminal Sacrifice	No Abnormality Detected
	02	Female	Terminal Sacrifice	No Abnormality Detected
	03	Female	Terminal Sacrifice	No Abnormality Detected

Key: mg/kg = milligram/kilogram, g = gram

III CONCLUSION

Based on the above results, the median lethal dose of Sunergist (Epibrassinolide 0.01% Soluble Liquid) after single oral administration to female rats, observed over a period of 14 days is:

$LD_{50} \geq 5000$ mg/kg body weight

B.6.1.2. Dermal

Annex point	KCP 7.1.2/01
Reference:	ACUTE DERMAL TOXICITY STUDY IN RATS WITH SUNERGIST (EPIBRASSINOLIDE 0.01% SOLUBLE LIQUID)
Author(s), year:	[redacted] (2017)
Report/Doc. number::	6127 (527-001)
Guideline(s):	OECD No. 402 (1987), OPPTS 870.1000, OPPTS 870.1200
GLP:	Yes
Deviations from OECD 402 (1987):	none
Acceptability:	Yes

Executive summary

The study was performed to assess the dermal toxicological potential of the test item Sunergist (Epibrassinolide 0.01% Soluble Liquid) in an *in vivo* test using Wistar Rats.
Five male and five female Wistar Rats were treated by a single dermal application at the dose of 2000 mg/kg body weight. One day before treatment, the back of the animals was clipped with electric clipper, exposing an area of approximately 10 % of the total body surface. The skin reactions were assessed. All the treated animals appeared normal and no systemic or local signs of toxicity were observed from day 0 of observation period after treatment until the end of observation period (day 14). All the animals survived until the end of the experimental period.
No abnormalities were observed in any of the treated animals during necropsy at terminal sacrifice.
Based on these results, the median lethal dose of Sunergist (Epibrassinolide 0.01% Soluble Liquid), in male and female rats after a single dermal administration, observed over a period of 14 days, was estimated to exceed 2000 mg/kg bw.

- **Dermal LD_{50} Males > 2000 mg/kg bw**
  Females > 2000 mg/kg bw
  Combined > 2000 mg/kg bw

I. MATERIALS AND METHODS

A. Test material and vehicle

1 Test materials

Test substance	Sunergist (Epibrassinolide 0.01% SL)
CAS no.	78821-43-9
Lot/batch	002-20150506
Formulation Type	Epibrassinolide 0.01%; Surfactants and Solvents: 99.99%
Concentration	0.01 %
Expiry date	May 05, 2017
Storage conditions:	Room Temperature (20 °C to 30 °C)

2 Test animals

Species	Rat
Strain	Wistar Rats (*Rattus norvegicus)*
Age	11-12 weeks
Bodyweight at dosing	Males: 249.2 - 260.1 g Females: 220.8 - 229.8 g
Source	[redacted]
Acclimation period	6 days
Diet	*Ad libitum* Teklad Certified Global 14% Protein Rodent Maintenance Diet (Batch No.2014C-111215MA) from ENVIGO
Water	*Ad libitum* tap water
Housing	Individually
Temperature	20.5 - 22.9°C
Humidity	53 - 64%
Ventilation	≥ 10 times/hour
Photoperiod	12 hours light / 12 hours dark

B. Study design and method

Test substance preparation and administration

Five male and five female Wistar rats were treated with Sunergist by a single dermal application at the dose of 2000 mg/kg body weight. The test item was applied undiluted as delivered by the sponsor.

The weight per ml of the test item was determined before the study initiation date and the average weight per ml was found to be 959.33 mg/mL.

Since test item related mortality was not observed in any of the treated animals, the experiment was completed as limit test only. One day before treatment, the back of the animals was clipped (with electric clipper), exposing an area of approximately 10 % of the total body surface.

On test day 0, the test item was transferred to a surgical gauze patch and moistened with a minimum amount of distilled water and applied to the intact skin. This gauze patch was covered with a semi-occlusive dressing. The dressing was wrapped around the abdomen and anchored with non-irritating adhesive tape.

The application volume was 2.08 mL/kg (=2000 mg/kg ÷ 959.33 mg/mL).

After the 24-hour application period, the dressings were removed and the skin was gently wiped with cotton soaked in distilled water. The skin reactions were assessed.

Mortality and clinical observations

The animals were observed daily during the acclimatization period for mortality/viability. All the animals were observed for clinical signs during first 30 minutes and at approximately 1, 2, 3 and 4 hours post treatment on day 0 and once daily during test days 1-14. Mortality/viability was recorded during the first 30 minutes and at approximately 1, 2, 3 and 4 hours post application on test day 0 (in common with the clinical signs) and twice daily during days 1-14 (once on the day of sacrifice). Local signs/skin reactions were observed daily from test days 1-14 (in common with clinical signs).

Bodyweight

Body weights were recorded on days 0 (prior to application), 7 and 14.

Necropsy

All animals were sacrificed at the end of the observation period by carbon dioxide asphyxiation in euthanasia chamber and discarded after the gross/macroscopic pathological changes were observed and recorded. No organs or tissues were retained.

C. Statistics

Data were not analysed statistically.

II. RESULTS AND DISCUSSION

Mortality and clinical signs

All the treated animals appeared normal and no systemic or local signs of toxicity were observed from day 0 of observation period till the last day of observation period (day 14). All the animals survived till the end of the experimental period. No mortality was observed in any of the treated animals.

Table 6.1.2-1: Mortality / Clinical Signs/ Local Signs / Skin Reactions

Dose	Sex	Animal Number	Test day																	
			0*					1	2	3	4	5	6	7	8	9	10	11	12	13	14
			0.5	1	2	3	4														
2000 mg/kg body weight	Male	01	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
		02	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
		03	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
		04	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
		05	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
	Female	06	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
		07	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
		08	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
		09	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
		10	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1

Key: mg/kg = milligram/kilogram, 1 = Normal.

* Examinations/observations were performed during the first 0.5 hour and at approximately 1, 2, 3 and 4 hours after test item application on test day 0.

No clinical sings were evident in any animal during acclimatization.

Bodyweights
The body weight of the animals was within the normal range of variability commonly recorded for this species, strain and age. There was body weight gain in all the animals by days 7 and 14 when compared to day 0.

Table 6.1.2-2: Body Weights

Dose	Sex	Animal Number	Test day 0 (pre-treatment) (g)	Test day 7 (g)	Test day 14 (g)	Body Weight Gain (%) (Test day 0-7)	Body Weight Gain (%) (Test day 0-14)
2000 mg/kg body weight	Male	01	249.2	275.1	296.5	10.4	19.0
		02	259.9	284.2	304.7	9.3	17.2
		03	252.8	275.7	297.0	9.1	17.5
		04	259.7	283.9	305.5	9.3	17.6
		05	260.1	285.6	307.6	9.8	18.3
	Female	06	220.8	236.8	250.2	7.2	13.3
		07	222.4	238.3	252.6	7.1	13.6
		08	229.8	240.0	255.9	4.4	11.4
		09	223.4	236.3	251.6	5.8	12.6
		10	229.2	244.8	258.2	6.8	12.7

Key: mg/kg = milligram/kilogram, g = gram

Necropsy
No abnormalities were detected in any of the treated animals during necropsy at terminal sacrifice.

Table 6.1.2-3: Macroscopic findings

Dose	Sex	Animal Number	Mode of Death	Macroscopic Findings
2000 mg/kg body weight	Male	01	Terminal Sacrifice	No Abnormality Detected
		02	Terminal Sacrifice	No Abnormality Detected
		03	Terminal Sacrifice	No Abnormality Detected
		04	Terminal Sacrifice	No Abnormality Detected
		05	Terminal Sacrifice	No Abnormality Detected
	Female	06	Terminal Sacrifice	No Abnormality Detected
		07	Terminal Sacrifice	No Abnormality Detected
		08	Terminal Sacrifice	No Abnormality Detected
		09	Terminal Sacrifice	No Abnormality Detected
		10	Terminal Sacrifice	No Abnormality Detected

Key: mg/kg = milligram/kilogram

III CONCLUSION
The median lethal dose of Sunergist (Epibrassinolide 0.01% Soluble Liquid), following single dermal application in Wistar rats of either sexes, observed over a period of 14 days is:

$LD_{50} \geq 2000$ mg/kg body weight

B.6.1.3. Inhalation

For the determination of the acute inhalation toxicity of Sunergist (Epibrassinolide 0.01% Soluble Liquid), no new study is submitted. A study for the acute inhalation toxicity in rats of the active substance 24-Epibrassionlide has been conducted, revealing no toxicity with a LC50 greater than the maximum attainable concentration of 1.08 ug/kg. Co-formulants are not classified for inhalation toxicity (see confidential information).
Considering animal welfare and the overall low toxicity of the active substance, as well as the widespread natural occurrence, no new study has been conducted.

B.6.1.4. Skin irritation

Annex point	KCP 7.1.4/01
Reference:	ACUTE DERMAL IRRITATION/CORROSION STUDY IN RABBITS WITH SUNERGIST (EPIBRASSINOLIDE 0.01% SOLUBLE LIQUID)
Author(s), year:	[redacted] (2017)
Report/Doc. number::	6128 (565-002)
Guideline(s):	OECD No. 404 (2015), OPPTS 870.1000, OPPTS 870.2500
GLP:	Yes
Deviations from OECD 404 (2015):	none
Acceptability:	Yes

Executive summary
The acute dermal irritation/corrosion of Sunergist (Epibrassinolide 0.01% Soluble Liquid) in rabbits was investigated according to OECD test guideline Number 404.
A dose of 0.5 g of Sunergist (Epibrassinolide 0.01% Soluble Liquid) was applied topical to the left flank of three New Zealand White rabbit for the duration of 4 hours held by a gauze patch and bandaging.
After the exposure period, any remaining unabsorbed test item was removed. Each of the sites was examined and scored for signs of irritation at 1, 24, 48 and 72 hours after the removal of the bandaging and patch. Local effects at the application site were scored according to the Numerical Scoring System (OECD 404).
The test item did not induce any degree of erythema or oedema and no clinical sign were observed in any of the animals. No abnormalities were detected in any of the treated animals during necropsy at terminal sacrifice.

Therefore, Sunergist (Epibrassinolide 0.01 % Soluble Liquid) is classified as "**Not Irritating**" to the rabbit skin.

- **Non Irritating**

I. MATERIALS AND METHODS

A. Test material and vehicle

1 Test materials

Test substance	Sunergist (Epibrassinolide 0.01% SL)
CAS no.	78821-43-9
Lot/batch	002-20150506
Formulation Type	Epibrassinolide 0.01%; Surfactants and Solvents: 99.99%
Concentration	0.01 %
Expiry date	May 05, 2017
Storage conditions:	Room Temperature (20 °C to 30 °C)

2 Test animals

Species	Rabbit
Strain	Young Adult male New Zealand White Rabbits (*Oryctolagus cuniculus)*
Age	12 - 14 weeks
Bodyweight at dosing	2.0002 - 2.4710 Kg
Source	[redacted]
Acclimation period	6 – 9 days
Diet	*Ad libitum* Teklad Certified Global 14% Protein Rodent Maintenance Diet (Batch No.2014C-111215MA) from ENVIGO
Water	*Ad libitum* tap water
Housing	Individually
Temperature	21.2 - 22.7 °C
Humidity	55 - 67 %
Ventilation	≥ 10 times/hour
Photoperiod	12 hours light / 12 hours dark

B. Study design and method

Treatment

Approximately 24 (±2) hours before application of the test item, both flanks of the rabbits were clipped free of hair, exposing an area of approximately 100 cm^2. Care was taken to avoid abrading of the skin during the clipping and only animals without any skin injury or overt signs of irritation were used in the test.

Administration

On the day of treatment, 0.5 mL of test item was applied to the intact skin (approximately, 4 cm x 4 cm) of the clipped area and covered with gauze patch. The gauze loaded with test item was applied to the treatment site of the clipped area (left flank), while the control site (right flank) was left untreated. The patches were covered with a semi-occlusive dressing. The dressing was wrapped around the abdomen and anchored with non-irritating adhesive tape.

After the 4-hour exposure period, the bandaging and patch were removed from each rabbit. The skin was gently wiped clean with cotton soaked in distilled water, to aid in the removal of any remaining test substance.

All animals were necropsied after 72 hour observation period.

Observations

Mortality

All animals were observed twice daily for mortality and viability, from commencement of acclimatization of the animals to the termination of the test.

Clinical signs

All animals were observed once daily for clinical signs, from commencement of acclimatization of the animals to the termination of the test.

Dermal Observation

Each test site was evaluated and scored for erythema, eschar and oedema and he skin reaction was assessed according to the numerical scoring system listed in the OECD guidelines No. 404, "Grading of Skin Reactions" (28th July 2015), at approximately 1, 24, 48 and 72 hours post removal of the dressing, gauze patch and unabsorbed test item.

To evaluate the irritation potential of the test item the mean score was calculated across 3 scoring intervals (24, 48 and 72 hours, after patch removal) for each animal for erythema/eschar grades and for oedema grades, separately and classified according to the classification criteria

Bodyweight

Bodyweight was assessed on the day of commencement of acclimatization, test item application and at termination of observation.

Necropsy

All animals were sacrificed at the end of the observation period and discarded after the gross/macroscopic pathological changes were observed and recorded.

No organs or tissues were retained.

C. Statistics

Data were not analysed statistically.

I. RESULTS AND DISCUSSION

Mortality and clinical signs

No mortality occurred during the acclimatization and experimental periods.

No clinical signs were observed in any of the animals throughout the acclimatization and experimental periods respectively.

Table 6.1.4-1: Mortality / Clinical Signs

Animal No.	Sex	Test day*							
		0		1		2		3	
		M	E	M	E	M	E	M	E
01	Male	1	1	1	1	1	1	1#	-

02		1	1	1	1	1	1	1#	-
03		1	1	1	1	1	1	1#	-

Key: M= Morning check, E= Evening check, 1= Normal.
*Examinations were performed twice daily.
No clinical signs were evident in any animal during the acclimatization.
Animal necropsied after 72 hour.

Dermal Observation
All individual mean scores for erythema/eschar formation for Animal No. 01, 02 and 03 at 24, 48 and 72 hours were 0.00. All individual mean scores of oedema for Animal No. 01, 02 and 03 at 24, 48 and 72 hours were also 0.00. The test item did not result in any coloration on the treated skin of any rabbit and no corrosive effects were observed on the skin of treated animals.

Table 6.1.4-2: Skin irritation Scores – Individual Values – Right Flank (Control)

Skin Reactions	**Animal Number**											
	01				**02**				**03**			
	At hour				**At hour**				**At hour**			
	1	**24**	**48**	**72**	**1**	**24**	**48**	**72**	**1**	**24**	**48**	**72**
Erythema	0	0	0	0	0	0	0	0	0	0	0	0
Oedema	0	0	0	0	0	0	0	0	0	0	0	0

Table 6.1.4-3: Skin irritation Scores – Individual Values – Left Flank (Treated)

Skin Reactions	**Animal Number**											
	01				**02**				**03**			
	At hour				**At hour**				**At hour**			
	1	**24**	**48**	**72**	**1**	**24**	**48**	**72**	**1**	**24**	**48**	**72**
Erythema	0	0	0	0	0	0	0	0	0	0	0	0
Oedema	0	0	0	0	0	0	0	0	0	0	0	0

Bodyweights
The body weights of all the animals were considered to be within the normal range of variability commonly observed for this species, stain and age.

Table 6.1.4-4: Body Weight - Individual

Body weight in Kg				
Animal Number	**Sex**	**First Day of Acclimatization**	**Day of Treatment**	**Last Day of Observation**
01	Male	2.3719	2.4710	2.5417
02		2.1785	2.2438	2.3204
03		1.7974	2.0002	2.0749

Necropsy
No abnormalities were detected in any of the treated animals during necropsy at terminal sacrifice.

III. CONCLUSION

Sunergist (Epibrassinolide 0.01% Soluble Liquid) is classified as **"Non Irritant"** to the rabbit skin.

B.6.1.5. Eye irritation

Annex point	KCP 7.1.5/01
Reference:	ACUTE EYE IRRITATION/CORROSION STUDY IN RABBITS WITH SUNERGIST (EPIBRASSINOLIDE 0.01% SOLUBLE LIQUID)
Author(s), year:	[redacted] (2017)
Report/Doc. number::	6129 (566-002)
Guideline(s):	OECD No. 405 (2012), OPPTS 870.1000, OPPTS 870.2400
GLP:	Yes
Deviations from OECD 405 (2012):	none
Acceptability:	Yes

Executive summary

The acute eye irritation/corrosion study of Sunergist (Epibrassinolide 0.01% Soluble Liquid) in rabbits was investigated according to OECD test guideline 405. Approximately 0.1 mL of test item was placed in the conjunctival sac of the left eye of male rabbits.
The scoring of eye reactions was performed at 1, 24, 48 and 72 hours after test item instillation. The mean score was calculated across 3 scoring times (24, 48 and 72 hours after treatment) for each animal for corneal opacity, lesions in iris, conjunctival redness and chemosis. The individual mean score of opacity, lesions in iris, conjunctival redness and chemosis for Animal No. 01, 02 and 03 were 0.00, 0.00, 0.33 and 0.00, respectively.
In Animal No. 01, 02 and 03 instillation of the test item did not show any degree of opacity, lesion in iris and chemosis throughout the study period.
No abnormalities were detected in any of the treated animals during necropsy at terminal sacrifice.

- **Not eye irritant**

III. MATERIALS AND METHODS

B. Test material and vehicle

1 Test materials

Test substance	Sunergist (Epibrassinolide 0.01% SL)
CAS no.	78821-43-9
Lot/batch	002-20150506
Formulation Type	Epibrassinolide 0.01%; Surfactants and Solvents: 99.99%
Expiry date	May 05, 2017
Storage conditions:	Room Temperature (20 °C to 30 °C)
Concentration	0.01 %

2 Test animals

Species	Rabbit
Strain	Young Adult male New Zealand White Rabbits (Oryctolagus cuniculus)
Age	12 - 14 weeks
Bodyweight at dosing	2.3984 - 2.4874 Kg
Source	[redacted]
Acclimation period	6 days
Diet	*Ad libitum* Teklad Certified Global 14% Protein Rodent Maintenance Diet (Batch No.2014C-111215MA) from ENVIGO
Water	*Ad libitum* tap water
Housing	Individually
Temperature	20.9 - 22.4°C
Humidity	54 - 64 %
Ventilation	≥ 10 times/hour
Photoperiod	12 hours light / 12 hours dark

B. Study design and method

Test substance preparation
A formulation of 1 % (w/v) aqueous suspension was prepared. The average pH was found to be 6.55 (from Solubility and pH Test).

Pre treatment
Approximately 60 minutes prior to test item instillation, Buprenorphine 0.01 mg/kg was administered by subcutaneous injection and 5 minutes prior to test item instillation, two drops of topical ocular anesthetic (0.5% Proparacaine hydrochloride) was instilled in each eye. The eye (right eye) not treated with test item, but treated with topical anaesthetics served as control.

Post treatment
Buprenorphine 0.01 mg/kg and Meloxicam 0.5 mg/kg was administered by subcutaneous injection approximately eight hours after test item instillation. After the initial 8-hour post treatment, Buprenorphine 0.01 mg/kg was administered by subcutaneous injection to the animals every 12 hours, in conjunction with Meloxicam 0.5 mg/kg every 24 hours up to 72 hour for all the treated animals.

Main test
Treatment
On the day of treatment, approximately 0.1 mL of the test item was placed in the conjunctival sac of the left eye of each animal after gently pulling the lower lid away from the eyeball. The lids were then gently held together for about one second to prevent loss of test item. The right eye remained untreated and served as control.
A single male rabbit was treated first. As no severe eye reactions were observed up to approximately 48 hours post exposure, the test was completed using the remaining two male rabbits. The treated eyes were washed with distilled water at approximately 24 hours after instillation.

Mortality and clinical observations
Mortality and Viability was observed at least twice daily from commencement of acclimatization of the animals to the termination of the test.
Clinical signs were observed once daily from commencement of acclimatization of the animals and twice daily with a minimum of 6 hours between observations from the test item instillation of the animal to the termination of the test.

Ocular observations
The eye reactions were assessed according to the numerical scoring system listed in the OECD guidelines No. 405, “Grading of Ocular Lesions” (02nd October, 2012). The eyes were examined at approximately 1, 24, 48 and 72 hours post instillation of test item. Fluorescein sodium ophthalmic strip and Neitz ophthalmoscope (Model; BX α-13) were used for scoring of eye lesions of all the rabbits.
Data is summarized in tabular form, for each individual animal the irritation scores for the designated observation time, a description of the degree and nature of irritation, the presence of serious lesions and non-ocular effects. The scores of each animal at the recording interval (24, 48, 72 hours) were used in calculating the respective mean values for each type of lesion.

Bodyweight
Body weights were assessed on the day of commencement of acclimatization, test item instillation and at termination of observation.

Necropsy
All animals were sacrificed at the end of the observation period by intravenous injection of thiopentone sodium at a dose of at least 1.0 ml/kg body weight (equivalent to 100 mg thiopentone sodium/kg body weight) and discarded after the gross/macroscopic pathological changes are observed and recorded. No organs or tissues were retained.

C. Statistics
No statistical analysis was performed.

I. RESULTS AND DISCUSSION

Mortality and clinical signs
No mortalities were observed during the experimental period.

No clinical signs suggestive of systemic toxicity were observed in any of the animals throughout the acclimatization and post treatment periods.

Ocular observations
The scoring of eye reactions was performed at 1, 24, 48, 72 hours, for Animal No. 01, 02 and 03 post test item instillation. The mean score was calculated across 3 scoring times (24, 48 and 72 hours after treatment) for each animal for corneal opacity, lesions in iris, conjunctival redness and chemosis. The individual mean score of opacity, lesions in iris, conjunctival redness and chemosis for Animal No. 01, 02 and 03 were 0.00, 0.00, 0.33 and 0.00, respectively .

Table 6.1.5-1: Eye Irritation Scores (treated - left eye)

Sex	**Male**														
Animal No.	**01**					**02**					**03**				
Eye Reactions	**At hour**					**At hour**					**At hour**				
	PE	**1**	**24**	**48**	**72**	**PE**	**1**	**24**	**48**	**72**	**PE**	**1**	**24**	**48**	**72**
Corneal Opacity	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Area of Opacity	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Iris	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Conjunctivae	0	1	1	0	0	0	1	1	0	0	0	1	1	0	0
Chemosis	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

Mean values			
Animal No.	01	02	03
Corneal Opacity	0.00	0.00	0.00
Iris	0.00	0.00	0.00
Conjunctivae	0.33	0.33	0.33
Chemosis	0.00	0.00	0.00

Key: PE = Pre Exposure, Note: For scoring

In Animal No. 01, 02 and 03 instillation of the test item did not show any degree of opacity, lesion in iris and chemosis throughout the study period. Some blood vessels observed at 1 and 24 hours were considered hyperaemic and appeared normal at 48 and 72 hours in all the animals**.**
The test item did not result in any coloration of the treated eyes of the rabbits.
No corrosive effects were observed on the cornea of the treated rabbits.

Bodyweights
The body weights of all the animals were considered to be within the normal range of variability commonly observed for this species, strain and age.

Table 6.1.5-2: Individual Animal Bodyweights

Animal Number	**Sex**	**First Day of Acclimatization (Kg)**	**Pre Treatment (Kg)**	**Pre Termination (Kg)**
01	Male	2.3166	2.4874	2.5369
02	Male	2.2850	2.4462	2.5059
03	Male	2.1856	2.3984	2.4548

Necropsy
No gross pathological abnormalities were observed in any of the treated animals during necropsy at terminal sacrifice.

Table 6.1.5-3: Gross / Macroscopic findings

Animal Number	Sex	Mode of death	Gross/Macroscopic Observations
01	Male	Terminal Sacrifice	No Abnormality Detected
02	Male	Terminal Sacrifice	No Abnormality Detected
03	Male	Terminal Sacrifice	No Abnormality Detected

Key: NAD – No Abnormality Detected

III CONCLUSION

Sunergist (Epibrassinolide 0.01% Soluble Liquid) is classified as **"Not Irritating"** to rabbit eyes.

B.6.1.6. Skin sensitization

Annex point	KCP 7.1.6/01
Reference:	CONTACT HYPERSENSITIVITY IN ALBINO GUINEA PIGS, MAXIMIZATION TEST (MAGNUSSON AND KLIGMAN METHOD) WITH WITH SUNERGIST (EPIBRASSINOLIDE 0.01% SOLUBLE LIQUID)
Author(s), year:	[redacted] (2017)
Report/Doc. number::	6130 (567-002)
Guideline(s):	OECD No. 406 (1992), OPPTS 870.1000, OPPTS 870.2600
GLP:	Yes
Deviations from OECD 406 (1992):	none
Acceptability:	Yes

Executive summary

In order to assess the allergenic potential of Sunergist (Epibrassinolide 0.01% Soluble Liquid), the Maximization Test was performed in 10 treated (Male) and 5 control (Male) Albino Dunkin Hartley Guinea Pigs, in accordance with OECD Guideline No. 406.

The intradermal induction of sensitization in the test group was performed in the dorsal skin from the scapular region with a 10% dilution of the test item in propylene glycol and in an emulsion of Freund's Complete Adjuvant (FCA)/Physiological saline. One day before the epidermal induction, the scapular region was painted with 0.5 mL of 10% sodium lauryl sulphate in Vaseline, in order to create local irritation. The epidermal induction of sensitization was conducted for 48 hours under occlusion with the test item at 100% one week after the intradermal induction. The animals of the control group were intradermally induced with propylene glycol and FCA/Physiological saline and epidermally induced with distilled water under occlusion.
Two weeks after epidermal induction the test and the control animals were challenged by epidermal application of the test item at 100% and distilled water under occlusion. Cutaneous reactions were evaluated at 24 hour and 48 hours after removal of the dressing.

Based on this results in an adjuvant sensitization test (M&K-test) in guinea pigs,
Sunergist (Epibrassinolide 0.01% Soluble Liquid)is considered to be **non-sensitising** (male).

III. MATERIALS AND METHODS

B. Test material and vehicle

1 Test materials

Test substance	Sunergist (Epibrassinolide 0.01% SL)
CAS no.	78821-43-9
Lot/batch	002-20150506
Formulation Type	Epibrassinolide 0.01%; Surfactants and Solvents: 99.99%
Concentration	0.01 %
Expiry date	May 05, 2017
Storage conditions:	Room Temperature (20 °C to 30 °C)

Vehicle	Propylene glycol (intradermal induction) Distilled water (epidermal induction)

2 Test animals

Species	Guinea Pig
Strain	Male Albino Dunkin Hartley Guinea Pig (*Cavia porcellus)*
Age	8 - 10 weeks
Bodyweight at dosing	299.2 – 341.4 g
Source	[redacted]
Acclimation period	5 days
Diet	*Ad libitum* Teklad Certified Global 14% Protein Rodent Maintenance Diet (Batch No.2014C-111215MA) from ENVIGO
Water	*Ad libitum* tap water
Housing	Individually
Temperature	20 ± 3°C
Humidity	30-70 %
Ventilation	≥ 10 times/hour
Photoperiod	12 hours light / 12 hours dark

B. Study design and method

Test substance preparation and administration

For the intradermal injection, an amount of 1000.07 mg of test item was mixed with Propylene Glycol and the final volume was made up to 10 ml. Thus, the concentration was 10%.
For the epidermal application, 0.2 mL of 100% test item was applied as such.
For the challenge application, 0.2 mL 100% test item was applied as such.

Intradermal Induction (Day 0)

An area of dorsal skin from the scapular region (approximately 6 x 8 cm) was clipped with a clipper or depilated with an approved depilatory cream. Three pairs of intradermal injections (0.1 mL/site) were given just within the boundaries of a 4 x 6 cm area in the cleared region as follows:

Test Group

Injection 1	1:1 (v/v) mixture of Freund's Complete Adjuvant and physiological saline.
Injection 2	The test item in its required concentration (10 %) is selected in Propylene glycol (w/v).
Injection 3	The test item at the selected concentration (10 %) in Propylene glycol formulated in a 1:1 (v/v) mixture of Freund's Complete Adjuvant and physiological saline (1:1 (v/v) mixture of Injection 1 and injection 2).

Control Group

Injection 1	1:1 (v/v) mixture of Freund's Complete Adjuvant and physiological saline.
Injection 2	Propylene glycol.
Injection 3	A 50% v/v formulation of the vehicle in a 1:1 (v/v) mixture of Freund's Complete Adjuvant and physiological saline (1:1 (v/v) mixture of Injection 1 and injection 2).

Injections 1 and 2 were given close to each other and nearest the head, while 3 was given towards the caudal part of the test area.
The skin reaction of this intradermal induction was observed at 24 (± 2) hours after intradermal injection according to the Magnusson and Kligman grading scale.

Epidermal Applications of Sodium Lauryl Sulphate (Day 6)

On day 5 (24 hours prior to the application), the right scapular region of the animals (approximately 6 x 8 cm) of all the guinea pigs were clipped with an electrical clipper. On day 6, the scapular region was painted with 0.5 ml of 10% sodium lauryl sulphate in Vaseline, in order to create a local irritation for control and treated animals.

Epidermal Application (Day 7)

On test day 7, one week after the intradermal injections, approximately, 2 x 4 cm patch of filter paper was saturated with 0.2 ml of 100% test item as such was placed over the right scapular area of the test animals.

The patch was covered by aluminium foil and secured with an adhesive tape. The dressings were removed after an exposure period of approximately 48 hours.
The guinea pigs of the control group were treated with distilled water only on the scapular region.
The reaction sites were assessed 24 hours after removal of the bandage for erythema and oedema according to the method of Magnusson and Kligman.

First Challenge (Day 21)
The test and the control guinea pigs were challenged two weeks after the epidermal induction application. Hair was removed with the help of a clipper from an area of approximately 5 x 5 cm on the left and right flank of each guinea pig 24 hours prior to the application. Two patches (approximately 3 x 3 cm) of filter papers, saturated with 0.2 ml of 100% non-irritating concentration of test item was applied to the left flank, while distilled water was applied on the right flank for all the animals using the same method as for the epidermal application. The dressings were left in place for approximately 24 hours.
Approximately 48 hours from the start of the challenge application, the skin reaction was observed and recorded according to the numerical grading system.
Approximately 24 hours after this observation a second observation (72 hours from the start of the challenge application) was made and once again recorded.

Rating of Allergenicity
Rating of Allergenicity has been conducted according to Magnusson and Kligman (1969):
Based upon the percentage of animals sensitized (24 and 48 hour reading), the test item will be assigned to one of the following five grades of allergenic potency according to Magnusson and Kligman, ranging from weak to extreme:

Sensitization Rate (%)	Grade	Classification
0 - 8	1	Weak
9 - 28	2	mild
29 - 64	3	moderate
65 - 80	4	strong
81 - 100	5	extreme

Mortality and clinical observations
The guinea pigs were observed twice daily from delivery of the animals to the termination of the test. Clinical signs have been observed daily.

Bodyweight
Body weight was measured on the day of acclimatization start, during randomization, before treatment and at the end of the experiment.

Necropsy
All surviving animals were sacrificed at the end of the observation period by carbon dioxide asphyxiation in euthanasia chamber and discarded after the gross/macroscopic pathological changes were observed and recorded. No organs or tissues will be retained.

C. Statistics
Descriptive statistics (mean and standard deviation) were calculated for body weights. No inferential statistics were used.

II. RESULTS AND DISCUSSION
III.

After intradermal induction
The findings at 24 hour observation after intradermal injection were, 6 out of 10 animals showed discrete erythema in the treated group. There were no findings in the control animals.

Table 6.1.6-1: Skin reaction after intradermal injection

Animal Number	Sex	REACTION AFTER INJECTION READINGS	
		24 hours at left scapular region	24 hours at right scapular region

Animal Number	Sex	REACTION AFTER INJECTION READINGS	
		24 hours at left scapular region	24 hours at right scapular region
09	Male	1	1
10	Male	1	1
11	Male	0	0
12	Male	1	1
13	Male	0	0
14	Male	1	1
15	Male	0	0
16	Male	1	1
17	Male	1	1
18	Male	0	0

After epidermal induction
The findings at 24 hour observation after patch removal were, 4 out of 10 animals showed positive skin reactions in the treated group. There were no findings in the control group.

Table 6.1.6-2: Skin reaction after epidermal injection

Animal Number	Sex	REACTION AFTER REMOVAL OF BANDAGE READINGS
		24 hours at scapular region
09	Male	0
10	Male	1
11	Male	0
12	Male	0
13	Male	1
14	Male	0
15	Male	0
16	Male	1
17	Male	1
18	Male	0

After epidermal challenge
No positive skin reactions were observed in the animals when treated with the test item at 100% concentration on left flank at 24 and 48 hour observations after patch removal.

Table 6.1.6-3: Skin Reactions after the Challenge Procedure (Male)

	after 24 hours	after 48 hours
	positive / total	positive / total

	% positive of total	% positive of total
CONTROL GROUP		
Sunergist (Epibrassinolide 0.01% Soluble Liquid), (100%) (left flank)	0 / 5 0	0 / 5 0
Distilled water only (right flank)	0 / 5 0	0 / 5 0
TREATMENT GROUP		
Sunergist (Epibrassinolide 0.01% Soluble Liquid), (100%) (left flank)	0 / 10 0	0 / 10 0
Distilled water only (right flank)	0 / 10 0	0 /10 0

No toxic signs were evident in the guinea pigs of the control or test groups.
No mortalities were observed during the course of the experiment.
No skin reactions were observed in the animals from the control and test group.

The results obtained from test animals following the challenge application was compared with the results seen in control animals.
No allergic reaction was found at the challenge site.
The test animals showed no evidence of contact hypersensitivity as there was no dermal reaction resulting from the challenge application in the treated group.
For evaluation of the incidence index both test and control animals were used. The incidence index is an expression of the number of animals showing a response of grade 1 or greater at the 24 and 48 hour reading out of the total animals in the group (this is expressed in percent).

Mortality and clinical signs
No mortality was observed in any of the treated animals during the experimental period.
No signs of systemic toxicity were observed in the animals either from the control or treated group.

Bodyweights
Normal body weight gain was observed in all the animals by the end of the experimental period.

Table 6.1.6-4: Summary of Body Weights

Sex	**Group**	**Mean ± Standard Deviation**	
		Pre Treatment	**Pre Sacrifice**
Male	Control Group	326.64 ± 9.025 n = 5	374.84 ± 9.643 n = 5
	Test Group	326.35 ± 10.879 n = 10	375.15 ± 10.828 n = 10

Key: n = Number of Animals

Necropsy
No abnormalities were detected in any of the treated animals during necropsy at terminal sacrifice

III. CONCLUSION

Based on the above findings in an adjuvant sensitization test (M&K-test) in male guinea pigs Sunergist (Epibrassinolide 0.01% Soluble Liquid) is considered to be **non-sensitising**.

B.6.1.7. Supplementary studies on the plant protection product

None available

B.6.1.8. Supplementary studies for combinations of plant protection products

None available

B.6.2. DERMAL ABSORPTION

No studies on dermal absorption have been submitted.

EFSA Panel on Plant Protection Products and their Residues (PPR); Guidance on Dermal Absorption. EFSA Journal 2012;10(4):2665. [30 pp.] doi:10.2903/j.efsa.2012.2665 was valid at the time of dossier submission (April 2017).

Therefore, the default values applicable for exposure estimations (no reference values were derived) would be **75%** (0,01% active ingredient in the formulation, MW 480,68 g/mol), log Pow = 2).

If default values proposed in the up-dated guidance document *EFSA (European Food Safety Authority), Buist H, Craig P, Dewhurst I, Hougaard Bennekou S, Kneuer C, Machera K, Pieper C, Court Marques D, Guillot G, Ruffo F and Chiusolo A, 2017. Guidance on dermal absorption. EFSA Journal 2017;15(6):4873, 60 pp. https://doi.org/10.2903/j.efsa.2017.4873* were applied **50%** dermal absorption would be considered appropriate for concentrate and in use dilution (water-based, low concentration of active ingredient).

B.6.3. AVAILABLE TOXICOLOGICAL DATA RELATING TO CO-FORMULANTS

Toxicological data on co-formulants is presented in the confidential part. No risk for human health is expected from the toxicological properties of the co-formulants at the concentrations present in the formulation.

B.6.4. EXPOSURE DATA

B.6.4.1. Operator exposure

The plant protection product Sunergist (Epibrassinolide 0.01 % Soluble Liquid) is intended as an elicitor and plant activator indoor as well as outdoor. The general method for application is automated spraying.

Sunergist is a soluble liquid formulation containing 0.1 g/L 24-Epibrassinolide.

The uses displayed in the following table represent worst case conditions with regard to non-dietary exposure.

Table 6.4.1-1:: Summary of use pattern and EU zones

Crop (Greenhouse / Field)	**Pests or Group of pests controlled**	**Application rate [g as/ha] a) max. rate per appl. b) max. total rate per crop/season**	**Growth stage**	**Number of applications**
Wine grapes and table grapes (Field)	Elicitor *Botryotinia fuckeliana* (BOTRCI) *Grey mould*	a) 0.05 g/ha b) 0.15 g/ha	BBCH 15 - 85	3
Cucurbits (Greenhouse)	Plant activator Antistress activity	a) 0.05 g/ha b) 0.15 g/ha	BBCH 12 - 69	3

Neither an AOEL or ADI, nor an ARfD are considered necessary, as the available database does not indicate a toxicological concern.

24-Epibrassinolide is a naturally occurring substance, brassinosteroids are present in all plants, resulting in ubiquitous exposure to humans and other organisms though the food chain. Guideline studies on acute toxicity, demonstrated that Sunergist (24-Epibrassinolide 0.01 % Soluble Liquid) has a low overall acute toxicity and was neither a dermal or eye irritant nor a dermal sensitizer. Brassinosteroids are readily metabolized by plants to inactive forms and, therefore, the application of Brassinolides to crop plants as a plant growth stimulant is unlikely to increase levels of brassinosteroids in or on the treated plants.
As no reference values (e.g. for AOEL) are considered necessary, exposure assessments for operator, bystander, worker and resident are not relevant and therefore are not conducted.
At the use rate and dilution applied for, exposure estimates for operators are in the ppm (µg/kg bw) range.

B.6.4.2. Bystander and resident exposure

Not conducted, see B.6.4.1

B.6.4.3. Worker exposure

Not conducted, see B.6.4.1

B.6.5. EXPOSURE AND RISK ASSESSMENT

Not conducted, see B.6.4.1

B.6.6. REFERENCES RELIED ON

Data Point	Author(s)	Year	Title Compagny Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Data protection claimed Y/N	Justification if data protection is claimed	Owner	Previous evaluation
KCP 7.1.1/01	[redacted]	2017	ACUTE ORAL TOXICITY STUDY IN RATS WITH SUNERGIST (EPIBRASSINOLIDE 0.01% SOLUBLE LIQUID) Report No.: 6126 (526-001) [redacted] GLP, unpublished	Y	Y	New study necessary for the approval of 24-Epibrassinolide	Suntton GmbH	N
KCP 7.1.2/01	[redacted]	2017	ACUTE DERMAL TOXICITY STUDY IN RATS WITH SUNERGIST (EPIBRASSINOLIDE 0.01% SOLUBLE LIQUID) Report No.: 6127 (527-001) [redacted] GLP, unpublished	Y	Y	New study necessary for the approval of 24-Epibrassinolide	Suntton GmbH	N
KCP 7.1.4/01	[redacted]	2017	ACUTE DERMAL IRRITATION/CORROSION STUDY IN RABBITS WITH SUNERGIST (EPIBRASSINOLIDE 0.01% SOLUBLE LIQUID) Report No.: 6128 (565-002) [redacted] GLP, unpublished	Y	Y	New study necessary for the approval of 24-Epibrassinolide	Suntton GmbH	N
KCP 7.1.5/01	[redacted]	2017	ACUTE EYE IRRITATION/CORROSION STUDY IN RABBITS WITH SUNERGIST (EPIBRASSINOLIDE 0.01% SOLUBLE LIQUID) Report No.: 6129 (566-002) [redacted] GLP, unpublished	Y	Y	New study necessary for the approval of 24-Epibrassinolide	Suntton GmbH	N
KCP 7.1.6/01	[redacted]	2017	CONTACT HYPERSENSITIVITY IN ALBINO GUINEA PIGS, MAXIMIZATION TEST (MAGNUSSON AND KLIGMAN METHOD) WITH WITH SUNERGIST (EPIBRASSINOLIDE 0.01% SOLUBLE LIQUID) Report No.: 6130 (567-002) [redacted] GLP, unpublished	Y	Y	New study necessary for the approval of 24-Epibrassinolide	Suntton GmbH	N