European Commission

Draft Assessment Report prepared according to the Commission Regulation (EU) N° 1107/2009
and
Proposal for Harmonised Classification and Labelling (CLH Report) according to Regulation (EC) N° 1272/2008

24-EPIBRASSINOLIDE

Volume 1

Rapporteur Member State: Austria

Version History

When	What
2018/05	Initial DAR
2018/08	DAR-CLH report revised in line with requirements of ECHA following the accordance check

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Level 1

24-EPIBRASSINOLIDE

1 STATEMENT OF SUBJECT MATTER AND PURPOSE FOR WHICH THIS REPORT HAS BEEN PREPARED AND BACKGROUND INFORMATION ON THE APPLICATION

1.1 CONTEXT IN WHICH THIS DRAFT ASSESSMENT REPORT WAS PREPARED

1.1.1 Purpose for which the draft assessment report was prepared

The present dossier is submitted for the approval of the new active substance 24-Epibrassinolide in the context of the following regulations:

REGULATION (EC) No 1107/2009 OF THE EUROPEAN PARLIAMENT AND OF THE COUNCIL of 21 October 2009 concerning the placing of plant protection products on the market

COMMISSION REGULATION (EU) No 283/2013 of 1 March 2013 setting out the data requirements for active substances, in accordance with Regulation (EC) No 1107/2009 of the European Parliament and of the Council concerning the placing of plant

COMMISSION REGULATION (EU) No 284/2013 of 1 March 2013 setting out the data requirements for plant protection products, in accordance with Regulation (EC) No 1107/2009 of the European Parliament and of the Council concerning the placing of plant

For the purposes of the evaluation of the new active substance 24-Epibrassinolide Austria has been designated as the Rapporteur Member State (RMS).

Proposed classification and labelling:

Aquatic chronic 4	H413	H413: May cause long lasting harmful effects to aquatic life

1.1.2 Arrangements between rapporteur Member State and co-rapporteur Member State

n.a.

1.1.3 EU Regulatory history for use in Plant Protection Products

n.a

1.1.4 Evaluations carried out under other regulatory contexts

n.a.

1.2 APPLICANT INFORMATION

1.2.1 Name and address of applicant(s) for approval of the active substance

Name: Suntton GmbH
Member of Suntton companies

Address: WORLD TRADE CENTER
City Airport Bremen
Hermann-Köhl-Straße 7
28199 Bremen
Germany

Person to contact: [redacted]

Tel.: [redacted]

Fax: -

E-mail: [redacted]

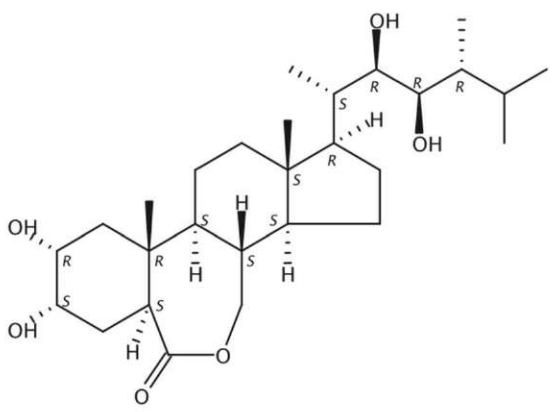
1.2.2 Producer or producers of the active substance

Confidential information, see Annex C.

1.2.3 Information relating to the collective provision of dossiers

Suntton is the sole applicant.

1.3 IDENTITY OF THE ACTIVE SUBSTANCE

1.3.1 Common name proposed or ISO-accepted and synonyms	**Common Name:** 24-Epibrassinolide No ISO name defined
1.3.2 Chemical name (IUPAC and CA nomenclature)	
IUPAC	(3aS,5S,6R,7aR,7bS,9aS,10R,12aS,12bS)-10-[(2S,3R,4R,5R)-3,4-dihydroxy-5,6-dimethylheptan-2-yl]-5,6-dihydroxy-7a,9a-dimethylhexadecahydro-3H-benzo[c]indeno[5,4-e]oxepin-3-one
CA	-
1.3.3 Producer's development code number	none
1.3.4 CAS, EEC and CIPAC numbers	
CAS	78821-43-9
EEC	-
CIPAC	-
1.3.5 Molecular and structural formula, molecular mass	
Molecular formula	$C_{28}H_{48}O_6$
Structural formula	
Molecular mass	480.7 g/mol

1.3.6 Method of manufacture (synthesis pathway) of the active substance	CONFIDENTIAL information - data provided separately in Volume 4
1.3.7 Specification of purity of the active substance in g/kg	min. 900 g/kg
1.3.8 Identity and content of additives (such as stabilisers) and impurities	
1.3.8.1 Additives	CONFIDENTIAL information - data provided separately in Volume 4
1.3.8.2 Significant impurities	CONFIDENTIAL information - data provided separately in Volume 4
1.3.8.3 Relevant impurities	none
1.3.9 Analytical profile of batches	CONFIDENTIAL information - data provided separately in Volume 4

1.4 INFORMATION ON THE PLANT PROTECTION PRODUCT

1.4.1 Applicant	(1) Suntton Co., Ltd. *or* (2) Sunergist Co., Ltd. Flat/Rm 1501(697) 15F, SPA Centre 53-55 Lockhart Road, Wanchai, Hong Kong	
1.4.2 Producer of the plant protection product	Confidential information, see Annex C.	
1.4.3 Trade name or proposed trade name and producer's development code number of the plant protection product	Sunergist	
1.4.4 Detailed quantitative and qualitative information on the composition of the plant protection product		
1.4.4.1 Composition of the plant protection product	24-Epibrassinolide 0.1 g/L	
1.4.4.2 Information on the active substances	**Type**	**Name/Code Number**
	ISO common name	24-Epibrassinolide
	CAS No	78821-43-9
	EC No	-
	CIPAC No	-
	Salt, ester anion or cation present	No
1.4.4.3 Information on safeners, synergists and co-formulants	CONFIDENTIAL information - data provided separately in Volume 4.	
1.4.5 Type and code of the plant protection product	soluble liquid [Code : SL]	

1.4.6 Function	Elicitor and plant activator
1.4.7 Field of use envisaged	Agriculture (viticulture, arable crops, and vegetable production)
1.4.8 Effects on harmful organisms	No direct fungicidal or any antagonistic effect against harmful organisms. 24-Epibrassinolide acts by activating and enhancing the defence and immune system of plants.

1.5 DETAILED USES OF THE PLANT PROTECTION PRODUCT

1.5.1 Details of representative uses

Crop and/or situation (a)	Member State or Country	Product name	F G or I (b)	Pests or Group of pests controlled (c)	Preparation		Application				Application rate per treatment			PHI (days) (m)	Remarks
					Type (d-f)	Conc. a.s. (i)	method kind (f-h)	range of growth stages & season (j)	number min-max (k)	Interval between application (min)	kg a.s /hL min-max (l)	Water L/ha min-max	kg a.s./ha min-max (l)		
Vitis vinifera VITVI (wine grapes and table grapes)	All Member states	Sunergist	F	Elicitor *Botryotinia fuckeliana* (BOTRCI) Grey mould	SL	0.099 g/L	Spraying	BBCH 15 to 85	3	7 days	1.5 - 2.5 mg a.s./hL	200 - 1000	0.05 g/ha	-	Volumes and doses will vary according to crop canopy size. Dilution rate: 1:2000
Vitis vinifera VITVI (wine grapes and table grapes)	All Member states	Sunergist	F	Plant activator Prevention environmental stress, quality and yield increase	SL	0.099 g/L	Spraying	BBCH 71-79	2	7 days	1.5 - 2.5 mg a.s./hL	200 - 1000	0.05 g/ha	-	Volumes and doses will vary according to crop canopy size. Dilution rate: 1:2000 to 1:3000
Leaf vegetables e.g. *Lactuca* sp. LACSS (Lettuce LACSA, prickly lettuce LACSE), dandelion TAROF, endive CICEN, chicory CICIN	All Member states	Sunergist	F	Elicitor *Bremia lactucae* (BREMLA) Downy mildew	SL	0.099 g/L	Spraying	BBCH 10 to 41	2	7 days	1 - 2 mg a.s./hL	200 - 400	0.04 g/ha	-	Volumes and doses will vary according to crop canopy size. Dilution rate: 1:1000
Leaf vegetables e.g. *Lactuca* sp. LACSS (Lettuce LACSA, prickly lettuce LACSE), dandelion TAROF, spinach SPQOL, witloof CICIF, chard BEAVV, lamb's lettuce VLLLO, Italian corn salad VLLER, endive CICEN	All Member states	Sunergist	F	Elicitor *Thanatephorus cucumeris* (RHIZSO) Bottom rot of lettuce	SL	0.099 g/L	Spraying	BBCH 10 to 41	2	7 days	1 - 2 mg a.s./hL	200 - 400	0.04 g/ha	-	Volumes and doses will vary according to crop canopy size: Dilution rate: 1:1000

Crop and/or situation (a)	**Member State or Country**	**Product name**	**F G or I** (b)	**Pests or Group of pests controlled** (c)	**Preparation**		**Application**				**Application rate per treatment**			**PHI (days)** (m)	**Remarks**
					Type (d-f)	Conc. a.s. (i)	method kind (f-h)	range of growth stages & season (j)	number min-max (k)	Interval between application (min)	kg a.s /hL min-max (l)	Water L/ha min-max	kg a.s./ha min-max (l)		
Sugarbeet BEAVA	All Member states	Sunergist	F	Elicitor *Cercospora beticola* (CERCBE) Leaf spot of beet	SL	0.099 g/L	Spraying	BBCH 12-39	3	7 days	0.5 -2 mg a.s./hL	200-800	0.04 g/ha	-	Volumes and doses will vary according to crop canopy size. Dilution rate: 1:2000
Cucurbits 1CUCF/FFFKU e.g. Cucumber CUMSC, zucchini CUUPG, squash CUUPE, pumpkin CUUPM, melon CUMME, water melon CITLA	All Member states	Sunergist	G	Plant activator Antistress activity	SL	0.099 g/L	Spraying	BBCH 12 to 69	3	7 days	1.5 - 2.5 mg a.s./hL	200-1000	0.05 g/ha	-	Volumes and doses will vary according to crop canopy size. Dilution rate: 1:2000 to 1:3000
Leaf vegetables e.g. *Lactuca* sp. LACSS (Lettuce LACSA, prickly lettuce LACSE), dandelion TAROF, endive CICEN, chicory CICIN	All Member states	Sunergist	G	Elicitor *Bremia lactucae* (BREMLA) Downy mildew	SL	0.099 g/L	Spraying	BBCH 10 to 41	2	7 days	1 - 2 mg a.s./hL	200 - 400	0.04 g/ha	-	Volumes and doses will vary according to crop canopy size. Dilution rate: 1:1000
Leaf vegetables e.g. *Lactuca* sp. LACSS (Lettuce LACSA, prickly lettuce LACSE), dandelion TAROF, spinach SPQOL, witloof CICIF, chard BEAVV, lamb's lettuce	All Member states	Sunergist	G	Elicitor *Thanatephorus cucumeris* (RHIZSO) bottom rot of lettuce	SL	0.099 g/L	Spraying	BBCH 10 to 41	2	7 days	1 - 2 mg a.s./hL	200 - 400	0.04 g/ha	-	Volumes and doses will vary according to crop canopy size: Dilution rate: 1:1000

Crop and/or situation (a)	**Member State or Country**	**Product name**	**F G or I** (b)	**Pests or Group of pests controlled** (c)	**Preparation**		**Application**				**Application rate per treatment**			**PHI (days)** (m)	**Remarks**
					Type (d-f)	Conc. a.s. (i)	method kind (f-h)	range of growth stages & season (j)	number min-max (k)	Interval between application (min)	kg a.s /hL min-max (l)	Water L/ha min-max	kg a.s./ha min-max (l)		
VLLLO, Italian corn salad VLLER, endive CICEN															

(a) For crops, the EU and Codex classifications (both) should be taken into account; where relevant, the use situation should be described (e.g. fumigation of a structure)
(b) Outdoor or field use (F), greenhouse application (G) or indoor application (I)
(c) *e.g.* biting and sucking insects, soil born insects, foliar fungi, weeds
(d) *e.g.* wettable powder (WP), emulsifiable concentrate (EC), granule (GR)
(e) CropLife International Technical Monograph no 2, 6th Edition. Revised May 2008. Catalogue of pesticide
(f) All abbreviations used must be explained
(g) Method, e.g. high volume spraying, low volume spraying, spreading, dusting, drench
(h) Kind, *e.g.* overall, broadcast, aerial spraying, row, individual plant, between the plant- type of equipment used must be indicated
(i) g/kg or g/L. Normally the rate should be given for the active substance (according to ISO) and not for the variant in order to compare the rate for same active substances used in different variants (e.g. fluoroxypyr). **In certain cases, where only one variant is synthesised, it is more appropriate to give the rate for the variant (e.g. benthiavalicarb-isopropyl).**
(j) Growth stage range from first to last treatment (BBCH Monograph, Growth Stages of Plants, 1997, Blackwell, ISBN 3-8263-3152-4), including where relevant, information on season at time of application
(k) Indicate the minimum and maximum number of applications possible under practical conditions of use
(l) The values should be given in g or kg whatever gives the more manageable number (e.g. 200 kg/ha instead of 200 000 g/ha or 12.5 g/ha instead of 0.0125 kg/ha
(m) PHI - minimum pre-harvest interval

1.5.2 Further information on representative uses

Information should be provided like:
- Details on method of application for specialised applications e.g. soil fumigants;
- Details on number and timing of applications and duration of protection, in case the GAP table gives ranges;
- Necessary waiting period or other precautions to avoid phytotoxic effects on succeeding crops;
- Proposed instructions for use.

1.5.3 Details of other uses applied for to support the setting of MRLs for uses beyond the representative uses

No specific MRLs are needed. 24-Epibrassinolide should be included into Annex IV of Regulation (EC) No 396/2005.

1.5.4 Overview on authorisations in EU Member States

n.a.

Level 2

24-EPIBRASSINOLIDE

2 SUMMARY OF ACTIVE SUBSTANCE HAZARD AND OF PRODUCT RISK ASSESSMENT

A literature search for the active substance 24-Epibrassinolide was performed in accordance to the provisions of the EFSA Guidance "Submission of scientific peer-reviewed open literature for the approval of pesticide active substances under Regulation (EC) 1107/2009".

The results of the search have been documented in LITERATURE REVIEW REPORT - ACTIVE SUBSTANCE: 24-Epibrassinolide, Reisinger, T., Huber, L. (2017), Company Report Number PP309-00002.

A detailed description is available in e.g. in part B.6.10 of this document.

The objective of the literature search was the assessment of scientific peer-reviewed open literature published within the last 10 years and dealing with side-effects on health, the environment and non-target species for the active substance 24-Epibrassinolide.

Literature was searched accessing the databases: AGRICOLA, BIOSIS, CABA, EMBASE, ESBIOBASE, HCAPLUS, MEDLINE, PASCAL, PQSCITECH, TOXCENTER via the service provider STN-International. The search strategy was based on a single concept search (CAS number and chemical names).

The search has been carried out on 09.11.2016 (calendar week 45).

2.1 IDENTITY

2.1.1 Summary of identity

All points of the data requirements regarding Section 1 have been addressed and the information supplied is acceptable. The technical specification is provided in the confidential part (Volume 4).

The purity of the active substance is 900 g/kg

There are no relevant impurities in 24-Epibrassinolide technical.

2.2 PHYSICAL AND CHEMICAL PROPERTIES [EQUIVALENT TO SECTION 7 OF THE CLH REPORT TEMPLATE]

2.2.1 Summary of physical and chemical properties of the active substance

Table 1: Summary of physicochemical properties of the active substance

Property	Value	Reference	Comment (e.g. measured or estimated)
Physical state at 20°C and 101,3 kPa	solid	Gao, J. (2015)	-
Melting/freezing point	Melting point: 254.6–258.2 ° C (97.2% purity)	Gao, J. (2015)	measured
Boiling point	No boiling or decomposition of the test item could be observed up to 400 °C	Gao, J. (2015)	measured
Relative density	-	-	-
Vapour pressure	1.90 x 10^{-15} Pa at 20 °C (97.2% purity) 8.67 x 10^{-15} Pa at 25 °C (97.2% purity)	Gao, J. (2015)	Estimated (OECD 104)

Property	Value	Reference	Comment (e.g. measured or estimated)
Surface tension	68 mN/m at 20 °C (90 % saturated solution) (97.2% purity)	Gao, J. (2015)	measured
Water solubility	3.8 mg/L at 20 °C (pH 4.5) (97.2% purity)	Gao, J. (2015)	measured
Partition coefficient n-octanol/water	log P_{OW} = 2.0 at 30 °C (97.2% purity) No dissociation of 24-Epibrassinolide in the range of pH 1.77 –12.51.	Gao, J. (2015)	measured
Henry's law constant	2.4 x 10^{-13} Pa.m³.mol^{-1}	-	calculated
Flash point	-	-	-
Flammability	24-Epibrassinolide is not ignitable and can thus be considered as not flammable.	Gao, J. (2015)	measured
Explosive properties	Considering the structural formula and the negative oxygen balance 24-Epibrassinolide is considered non-explosive	Feyrer, A.; Goerg, J. (2017)	statement
Self-ignition temperature	-	-	-
Oxidising properties	Considering the structural formula and the negative oxygen balance 24-Epibrassinolide is considered non-oxidizing.	Feyrer, A.; Goerg, J. (2017)	statement
Granulometry	-	-	-
Solubility in organic solvents and identity of relevant degradation products	28.7 g/L in methanol <3.1 x 10-3 g/L in n-heptane 5.5 g/L in ethyl acetate 6.0 g/L in dichloromethane 18.0 g/L in acetone 19 x 10-3 g/L in toluene	Gao, J. (2015)	measured
Dissociation constant	pKa = . No acid or base properties were found in the range pH 1.77 –12.51. (97.2% purity)	Gao, J. (2015)	measured
Viscosity	-	-	-
Spectra (UV/VIS, IR, NMR, MS), molar extinction at relevant wavelengths, optical purity	(97.2% purity) pH 5.57: λ_{max} (nm) ε (L mol^{-1} cm^{-1}) 205 183 215 91 pH 0.6: λ_{max} (nm) ε (L mol^{-1} cm^{-1}) 200 245 pH 13.4: λ_{max} (nm) ε (L mol^{-1} cm^{-1}) 219 67	Gao, J. (2015)	measured

2.2.1.1 Evaluation of physical hazards [equivalent to section 8 of the CLH report template]

2.2.1.1.1 Explosives *[equivalent to section 8.1 of the CLH report template]*

Table 2: Summary table of studies on explosive properties

Method	Results	Remarks	Reference
Regulation (EC) No 440; A.14.	Not explosive	No test has been performed with the substance. Information on the substance is provided as set out in 1.1 of method A.14	Feyrer, A.; Goerg, J. (2017)

2.2.1.1.1.1 Short summary and overall relevance of the provided information on explosive properties
Considering the structural formula and the negative oxygen balance 24-Epibrassinolide is considered non-explosive

2.2.1.1.1.2 Comparison with the CLP criteria
Oxygen balance is less than -200

2.2.1.1.1.3 Conclusion on classification and labelling for explosive properties
Not explosive

2.2.1.1.2 Flammable gases (including chemically unstable gases) *[equivalent to section 8.2 of the CLH report template]*

Not relevant

2.2.1.1.3 Oxidising gases *[equivalent to section 8.3 of the CLH report template]*

Not relevant

2.2.1.1.4 Gases under pressure *[equivalent to section 8.4 of the CLH report template]*

Not relevant

2.2.1.1.5 Flammable liquids *[equivalent to section 8.5 of the CLH report template]*

Not relevant

2.2.1.1.6 Flammable solids *[equivalent to section 8.6 of the CLH report template]*

Table 3: Summary table of studies on flammable solids

Method	Results	Remarks	Reference
Regulation (EC) No 440; A.10.	Not flammable		Gao, J. (2015)

2.2.1.1.6.1 Short summary and overall relevance of the provided information on flammable solids
The substance cannot be ignited within 2 minutes.

2.2.1.1.6.2 Comparison with the CLP criteria
The substance cannot be ignited.

2.2.1.1.6.3 Conclusion on classification and labelling for flammable solids
Not flammable

2.2.1.1.7 Self-reactive substances *[equivalent to section 8.7 of the CLH report template]*

Not relevant

2.2.1.1.8 Pyrophoric liquids *[equivalent to section 8.8 of the CLH report template]*

Not relevant

2.2.1.1.9 Pyrophoric solids *[equivalent to section 8.9 of the CLH report template]*

Experience in manufacture or handling shows that the substance does not ignite spontaneously on coming into contact with air at normal temperatures

2.2.1.1.10 Self-heating substances *[equivalent to section 8.10 of the CLH report template]*

Table 4: Summary table of studies on self-heating substances

Method	Results	Remarks	Reference
differential scanning calorimetry	2 endothermic peaks were found up tho the melting point of the substance		Gao, J. (2015)

2.2.1.1.10.1 Short summary and overall relevance of the provided information on self-heating substances

Three endothermic peaks were found at 44.96 - 134.76 °C, 172.37 - 207.26 °C and 239.79 - 285.15 °C.
The substance melts at 254.6–258.2 ° C.
The substance is not oxidizing (see 2.2.1.1.13)

2.2.1.1.10.2 Comparison with the CLP criteria

Self-heating is the result of an exothermic reaction of a substance or mixture with the oxygen in the air.
As no exothermic reaction occurs until the substance melts, the substance can be regarded not self-heating

2.2.1.1.10.3 Conclusion on classification and labelling for self-heating substances

Not self-heating

2.2.1.1.11 Substances which in contact with water emit flammable gases *[equivalent to section 8.11 of the CLH report template]*

Not relevant

2.2.1.1.12 Oxidising liquids *[equivalent to section 8.12 of the CLH report template]*

Not relevant

2.2.1.1.13 Oxidising solids *[equivalent to section 8.13 of the CLH report template]*

Table 5: Summary table of studies on oxidising solids

Method	Results	Remarks	Reference
Regulation (EC) No 440; A.17.	Not oxidising	No test has been performed with the substance. Information on the substance is provided as set out in 1.1 of method A.17	Feyrer, A.; Goerg, J. (2017)

2.2.1.1.13.1 Short summary and overall relevance of the provided information on oxidising solids

Considering the structural formula and the negative oxygen balance 24-Epibrassinolide is considered non-oxidizing.

2.2.1.1.13.2 Comparison with the CLP criteria

The substance contains oxygen, fluorine or chlorine and these elements are chemically bonded only to carbon or hydrogen.

2.2.1.1.13.3 Conclusion on classification and labelling for oxidising solids

Not oxidising

2.2.1.1.14 Organic peroxides *[equivalent to section 8.14 of the CLH report template]*

Not relevant

2.2.1.1.15 Corrosive to metals *[equivalent to section 8.15 of the CLH report template]*

Not relevant

2.2.2 Summary of physical and chemical properties of the plant protection product

The appearance of the plant protection product Sunergist (24-Epibrassinolide 0.01% Soluble Liquid (SL)) is that of colourless clear liquid. It has neither explosive nor oxidising properties. No flash point could be determined until boiling at 92.7 °C. A 1% aqueous solution shows a pH value of 6.13, while the neat formulation has a pH of 5.5. The density was found to be 0.993 g/ml at 20 °C. Persistent foam was measured to be 36 ml after 1 min at a concentration of 0.1%v/v. Transparent, homogeneous and non-layered appearance after dilution of Sunergist (24-Epibrassionlide 0.01% SL) in water confirms dilution stability of the formulation.
Moreover, the product is stable at 54°C for two weeks (accelerated storage stability test), for 7 days at 0°C and for 2 years at 20°C.
Its technical characteristics are acceptable for a SL formulation.

Data Gap: no foaming stability was determined at the maximum concentration of 025%v/v.

2.3 DATA ON APPLICATION AND EFFICACY

2.3.1 Summary of effectiveness

The representative formulation Sunergist (active ingredient: 24-Epibrassinolide) is an elicitor of plant's self-defence mechanisms against fungal diseases on wine and table grapes, leaf vegetables (e.g. lettuce) and sugar beet. Moreover, Sunergist is a plant activator to protect plants against abiotic stresses and to improve plant health in wine and table grapes and cucurbits.
No direct fungicidal or antagonistic effect against harmful organisms occurs.

Elicitor uses:
BOTRCI on wine grapes and table grapes (VITVI):
One GEP (South zone; EPPO Mediterranean zone) and 3 non-GEP trials (Central zone, EPPO Maritime zone) are available.
Significant effects were demonstrated in non-GEP trials only. A numerically reduction of disease incidence and severity on table and wine grapes may be expected.

BREMLA and RHIZSO on lettuce (LACSA):
Two GEP field trials (Central zone, EPPO Maritime zone) were presented.
Against RHIZSO, Sunergist significantly increased the proportion of marketable heads, compared to the untreated control. Against BREMLA no significant effect was seen. In one trial a numerical benefit was recorded, whereas in the other negligible efficacy was seen.
At least a numerically reduction of RHIZSO and BREMLA severity may be expected.

CERCBE on sugar beet (BEAVA):
A single GEP field trial (South zone, EPPO Mediterranean zone) was presented.
Sunergist at 0.1 l/ha and at 0.2 l/ha achieved a significant effect on disease severity. No significant effect was recorded for disease incidence.
Significant reduction of CERCBE severity may be expected.

Across uses, disease reduction at least in terms of numbers was demonstrated. Efficacy is regarded to be sufficiently supported with data at the current level of active substance evaluation. However, to demonstrate an economically relevant benefit of the application as elicitor against BOTRCI on VITVI, against BREMLA and RHIZSO on LACSA, and against CERCBE on BEAVA, further data is needed for product authorization.

Plant activator uses:
Prevention of environmental stress, as well as quality and yield increase is claimed for VITVI, and cucurbits.
Prevention of environmental stress was not supported by data.

5 non-GEP grapevine trials from the US/California (appraised to be comparable to the EPPO Maritime zone) were presented. In 3 trials Sunergist applied at dilution rates of 1:1893 to 1:3785 increased the yield. In one trial the onset of ripening was promoted, and yield was increased at the highest dilution rate, whereas in the other trials a lower bunch weight and a reduced yield was observed at the same dilution rate.

Yield was also assessed in one Maritime zone wine grape non-GEP trial and one Mediterranean zone sugar beet GEP trial. The application of Sunergist increased yield in terms of numbers.

Regarding quality parameters of the yield, in two GEP trials (one grapevine, one sugar beet) slight and statistically insignificant differences between test product treated samples, and the untreated reference samples were seen. Also in non-GEP trials no constant adverse or positive effects were recorded.

No assessments of the quality fresh fruits or vegetables (taint) were carried out.

No assessments on cucurbits were carried out.

To confirm a positive effect of Sunergist on the yield, or on the quality of yield, further data is needed for product authorization.

2.3.2 Summary of information on the development of resistance

No EPPO-conform resistance risk analysis was presented. Even though BOTRCI is a high risk target organism which has already developed resistance against several mode of action substance groups, the applicants rationale can be agreed: Resistance development is not to be expected, since Sunergist resp. 24-Epibrassinolide has no direct fungicidal effect. Thus baseline sensitivity data, as well as a detailed resistance risk analysis, can be waived.

2.3.3 Summary of adverse effects on treated crops

Specific studies on adverse effects on field crops were not conducted.

Crop safety was assessed in preliminary and efficacy trials on **VITVI** in the EPPO Mediterranean zone, and the EPPO Maritime zone. No data from the EPPO South-east zone are available (EPPO North-east is not relevant for viticulture). 24-Epibrassinolide is a phytohormone. Therefore, in particular in case of perennial crops such as VITVI, the possible effect on the crop after repeated application across several years should be assessed.
Crop safety was demonstrated for field-grown **LACSA**, in the EPPO Maritime zone only. No field trials conducted in other EPPO zones (South-east, North-East) are available. Sunergist was also not assessed on LACSA in the green house, and was not assessed on **cucurbits** at all (Plant activator use).
BEAVA was assessed in a single EPPO Mediterranean zone trial. No field trials conducted in other EPPO zones (Maritime, South-east, North-East) are available.
In none of the trials crop damage was reported. However, to confirm crop safety of Sunergist at the applied doses and spray volumes for the Central zone, further data is needed.

Yield as well as **quality of yield** were assessed to address plant activator uses (see above).

The possible impact on **transformation processes** was not assessed. Sunergist is applied in all crops without a PHI, therefore residues both of the active substance as well as of the formulation on the harvested parts of the plant have to be expected. No assessments of physically processed fruits or vegetables (taint) were carried out.
No direct effect onto pure yeasts resp. malolactic bacteria during microbial fermentation is expected, however, quality parameters of grape must, and thus also of wine, may be affected. To demonstrate the neutral behavior of Sunergist on processing resp. fermentation, further product data is needed.

2.3.4 Summary of observations on other undesirable or unintended side-effects

No specific studies regarding observations on undesirable or unintended side-effects were conducted. However, no undesirable or unintended side-effects were observed in any of the preliminary and efficacy trials conducted.

Crop safety was not sufficiently supported for all applied uses/crops. However, effects on **succeeding crops** usually are assessed for herbicides only.

In particular in high growing crops spray drift onto adjacent crops is possible. To confirm crop safety of Sunergist also for **adjacent** crops, further product data are needed.

No adverse effects of Sunergist on beneficial or other non-target organisms were reported in the trials. Details on the possible effects on beneficial organisms and other non-target organisms are summarized in B.9 (Ecotoxicology).

2.4 FURTHER INFORMATION

2.4.1 Summary of methods and precautions concerning handling, storage, transport or fire

Handling
Avoid contact with skin and eyes.
Ensure adequate ventilation.
Keep away from sources of ignition.
At work do not eat, drink and smoke.

Keep away from food, drink and animal feeding stuffs.
Wash hands before breaks and at the end of workday.
Do not breathe dust.
Provide eye bath.

Storage
Store only in original container at cool and aired place.
Protect from heat and direct solar radiation.
Keep container tightly closed.

Transport
No hazardous material as defined by the transport regulations

Fire-Fighting Measures
Suitable Extinguishing Media:
Foam, carbon dioxide (CO2), dry chemical, water-spray
Extinguishing media which must not be used for safety reasons
Full water jet.
Special hazards arising from the substance or mixture
Fire may produce: Carbon dioxide (CO2), Carbon monoxide (CO). Inhalation of hazardous decomposition products can cause serious damage to health.
Advice for fire-fighters
Wear self-contained breathing apparatus and protective suit.

2.4.2 Summary of procedures for destruction or decontamination

Accidental spillages of Sunergist should be collected mechanically and contaminated areas should be rinsed with water in accordance with local and national regulations.

2.4.3 Summary of emergency measures in case of an accident

a) Containment of spillages
Accidental spillages of Sunergist should be collected mechanically and contaminated areas should be rinsed with water.
b) Decontamination of areas, vehicles and buildings
Accidental spillages of Sunergist should be collected mechanically and contaminated areas should be rinsed with water.
Product Disposal
Disposal in accordance with local regulations.
This product is to be brought to a properly certified waste site approved to handle energy wastes.
c) Disposal of damaged packaging, absorbents and other materials
Disposal in accordance with local regulations.
d) Protection of emergency workers and residents, including bystanders
Advice for non-emergency personnel:
Evacuate personnel to safe areas.
Advice for emergency responders
Use personal protective equipment.
Sweep up to prevent slipping hazard.
Prevent further leakage or spillage.
e) First aid measures
First aid measures are described in the following:

2.5 METHODS OF ANALYSIS

2.5.1 Methods used for the generation of pre-authorisation data

Adequate methods are available for the analysis of the active in all relevant compartments.

2.5.2 Methods for post control and monitoring purposes

No methods are required for post control and monitoring purposes

2.6 EFFECTS ON HUMAN AND ANIMAL HEALTH

2.6.1 Summary of absorption, distribution, metabolism and excretion in mammals *[equivalent to section 9 of the CLH report template]*

No guideline-compliant studies on ADME behaviour of 24-epibrassinolide have been conducted.
Several articles have been cited by the applicant illustrating that plant sterols are ubiquitously present in plants and thus, humans are continually exposed to them. Dedicated studies are not considered necessary since natural background exposure via food is considered higher than exposure through the use as a plant protection product.

Brassinosteroids are found throughout the plant kingdom and are widely distributed in lower and higher plants. Until today, 69 natural brassinosteroids have been identified and have been detected in all plant organs, including pollen, anthers, seeds, leaves, stems, roots, flowers, fruits and grains. Through the dietary intake of plants and plant products, humans and animals are constantly exposed to Brassinosteroids and their metabolites. Highest concentrations of brassinosteoids in food are found in vegetable oils and nuts (Ogbe RJ, 2015).
The highest concentrations of Brassinosteroids in plants are found in pollen, immature seeds and fruits at a range of 1–100 µg/kg fresh weight, while shoots and leaves usually contain lower amounts of 0.01–0.1 µg/kg fresh weight.

A review article published in 2015 summarizes much of the current knowledge on phytosterol occurrence in plants, metabolism in mammals, and their current use as food additives (Ogbe RJ, 2015), the section on absorption and metabolism from this review is copied here in this section:
…
Though various diets contain similar amounts of phytosterols and cholesterol, serum phytosterol concentrations are usually several hundred times lower than serum cholesterol levels in humans. It was reported that less than 10% of dietary phytosterols are systematically absorbed, in contrast to about 50 – 60% of dietary cholesterol. Like cholesterol, phytosterols are incorporated into mixed micelles before they are taken up by enterocytes. Once inside the enterocytes their systemic absorption is inhibited by the activity of efflux transporters, consisting of a pair of ATP-binding cassette (ABC) proteins known as ABCG5 and ABCG8. ABCG5 and ABCG8 each forms one half of a transporter that secretes phytosterols and unesterified cholesterol from the enterocyte into the interestinal lumen. Phytosterols are secreted back into the intestine by ABCG5/G8 transporters at a much greater rate than cholesterol, resulting in much lower intestinal absorption of dietary phytosterol than cholesterol. Within the enterocytes, phytosterols are not as readily esterified as cholesterol, so they are incorporated into chylomicrons at much lower concentrations. Those phytosterols that are incorporated into chylomicrons enter blood circulation and are taken up by the liver. Once inside the liver, phytosterols are metabolized into cholesterol and other metabolites, by the action of several enzymes and a key enzyme called cholesterol 7α-hydroxylase into bile acids, and rapidly secreted into bile by hepatic ABC G5/G8 transporters. This enzyme is a regulatory enzyme in bile acids biosynthesis. Even though cholesterol could also be secreted into bile, the rate of phytosterol secretion into bile is greater than cholesterol secretion. Therefore, the low serum concentrations of phytosterols compared to cholesterol can be explained by decreased intestinal absorption and increased excretion of phytosterols into bile.
…

Through the dietary intake of plants and plant products, humans and animals are constantly exposed to brassinosteroids and their metabolites. Harmful effects are not likely from the use of 24-epibrassinolide in agriculture. In the light of these considerations, unnecessary animal testing should be avoided. The available literature indicates that plant sterols are metabolized alongside with cholesterol to bile acids and rapidly excreted.

2.6.2 Summary of acute toxicity

2.6.2.1 Acute toxicity - oral route [equivalent to section 10.1 of the CLH report template]

Table 6: Summary table of animal studies on acute oral toxicity

Method, guideline, deviations if any	Species, strain, sex, no/group	Test substance	Dose levels, duration of exposure	Value LD_{50}	Reference
OECD 423 (2002) Acute Toxic Class Method, no deviations,	Rats, Wistar, females, 3/ group	24-epibrassinolide	Single dose	> 5000 mg/kg bw	[redacted] (2017)

Method, guideline, deviations if any	Species, strain, sex, no/group	Test substance	Dose levels, duration of exposure	Value LD_{50}	Reference
study acceptable					

2.6.2.1.1 Short summary and overall relevance of the provided information on acute oral toxicity

The study was performed to assess the oral toxicological potential of the test item 24-Epibrassinolide (TGAI) in an in vivo test using 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 a vehicle (refined groundnut oil) 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 24-Epibrassinolide (TGAI) after single oral administration to female rats, observed over a period of 14 days is:

- LD50 Females > 5000 mg/kg body weight

No reliable information on acute oral toxicity was retrieved from public literature.

2.6.2.1.2 Comparison with the CLP criteria regarding acute oral toxicity

For acute oral toxicity, the LD_{50} value observed in the available guideline-compliant study is higher than 5000 mg/kg bw.
Substances whose LD_{50} is ≥ 2000 mg/kg bw do not fulfil the criteria for classification as acutely toxic via the oral route according to the criteria in CLP Annex I, 3.1.2 to 3.1.3.4.

2.6.2.1.3 Conclusion on classification and labelling for acute oral toxicity

Conclusive and sufficient for classification. No classification proposed.

2.6.2.2 Acute toxicity - dermal route [equivalent to section 10.2 of the CLH report template]

Table 7: Summary table of animal studies on acute dermal toxicity

Method, guideline, deviations if any	Species, strain, sex, no/group	Test substance	Dose levels, duration of exposure	Value LD_{50}	Reference
OECD 402 (1987), no deviations, study acceptable	Rat, Wistar, males/females, 5/group	24-epibrassinolide	Single dose	> 2000 mg/kg bw	[redacted] (2017)

2.6.2.2.1 Short summary and overall relevance of the provided information on acute dermal toxicity

The study was performed to assess the dermal toxicological potential of the test item 24-Epibrassinolide (TGAI) 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 after treatment until the end of the 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.
Based on these results, the median lethal dose of 24-Epibrassinolide (TGAI) 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

No reliable information on acute dermal toxicity was retrieved from public literature.

2.6.2.2.2 Comparison with the CLP criteria regarding acute dermal toxicity

Substances whose LD_{50} is ≥ 2000 mg/kg bw do not fulfil the criteria for classification for acute toxicity via the dermal route according to the criteria in CLP Annex I, 3.1.2 to 3.1.3.4.

2.6.2.2.3 Conclusion on classification and labelling for acute dermal toxicity

Conclusive and sufficient for classification. No classification proposed.

2.6.2.3 Acute toxicity - inhalation route [equivalent to section 10.3 of the CLH report template]

Table 8: Summary table of animal studies on acute inhalation toxicity

Method, guideline, deviations if any	Species, strain, sex, no/group	Test substance, form and particle size (MMAD)	Dose levels, duration of exposure	Value LC_{50}	Reference
OECD 426 (2002), no deviations, study acceptable	Rat, Wistar, males/females, 3/group	24-epibrassinolide, dust, MMAD 2,54-3,01 µm	Single dose, 4 hours	> 1.08 mg/L	[redacted] (2017)

2.6.2.3.1 Short summary and overall relevance of the provided information on acute inhalation toxicity

The purpose of this study was to assess the acute inhalation toxicity of 24-Epibrassinolide in rats.
Three male and three female rats were exposed to 24-Epibrassinolide (TGAI) for a period of 4 hours/single exposure, at a concentration of 1.08 mg/L air (maximum attainable concentration).
To attain this nominal concentration, dust aerosol was generated from the test item using a rotating brush aerosol generator and piston speed was set at a rate 100 mL/min.
Attempt was made to achieve a higher concentration by increasing the speed set rate greater than 100 mL/min, but the generation of aerosol was poor at this speed. Hence, the concentration of 1.08 mg/L achieved at the speed rate of 100 mL/min was considered as the maximum attainable concentration.

After the treatment, the rats were observed for a total of 14 days. Mortality, clinical signs, bodyweights and feed and water consumption were recorded during the observation period and all animals were examined macroscopically at scheduled necropsy.
During exposure, no mortality was observed and all animals appeared normal throughout the experimental period.
No abnormalities were detected in any of the animals on necropsy at the end of observation period.

- LC_{50} Males > 1.08 mg/L (maximum attainable concentration)
  Females > 1.08 mg/L (maximum attainable concentration)
  Combined > 1.08 mg/L (maximum attainable concentration)

No reliable information on acute inhalation oxicity was retrieved from public literature.

2.6.2.3.2 Comparison with the CLP criteria regarding acute inhalation toxicity

Substances whose LD_{50} is ≥ 5 mg/L do not fulfil the criteria for classification for acute toxicity via the inhalation route according to the criteria in CLP Annex I, 3.1.2 to 3.1.3.4. No indications for inhalatory toxicity were observed at the maximum attainable concentration of 1.08mg/L.

2.6.2.3.3 Conclusion on classification and labelling for acute inhalation toxicity

Conclusive and sufficient for classification. No classification proposed.

2.6.2.4 Skin corrosion/irritation [equivalent to section 10.4 of the CLH report template]

Table 9: Summary table of animal studies on skin corrosion/irritation

Method, guideline, deviations if any	Species, strain, sex, no/group	Test substance	Dose levels, duration of exposure	Results - Observations and time point of onset - Mean scores/animal - Reversibility	Reference
OECD 405 (2012), no deviations, study acceptable	Rabbit, New Zealand White, males, 3/group	24-epibrassinolide	0,5g / animal, 4 hours	No effects observed Mean scores for all animals at all timepoints (erythema/oedema): 24 hours, animal 1: 0/ 0 48 hours, animal 2: 0/ 0 72 hours, animal 3: 0/ 0	[redacted] (2017)

2.6.2.4.1 Short summary and overall relevance of the provided information on skin corrosion/irritation

The study was performed to assess the dermal toxicological potential of the test item 24-Epibrassinolide (TGAI) in an *in vivo* test using rabbits.
The test item was applied by topical (semi-occlusive) application of 0.5 g to the intact skin of the left flank of male rabbits for an exposure period of 4 hours.
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). The test item did not induce any degree of erythema or oedema. 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, 24-Epibrassinolide (TGAI) is classified as "Non Irritant" to the rabbit's skin.

2.6.2.4.2 Comparison with the CLP criteria regarding skin corrosion/irritation

According to the criteria in CLP, this substance does not fulfil the criteria for classification as irritant to the skin.

2.6.2.4.3 Conclusion on classification and labelling for skin corrosion/irritation

Conclusive and sufficient for classification. No classification proposed.

2.6.2.5 Serious eye damage/eye irritation [equivalent to section 10.5 of the CLH report template]

Table 10: Summary table of animal studies on serious eye damage/eye irritation

Method, guideline, deviations if any	Species, strain, sex, no/group	Test substance	Dose levels duration of exposure	Results - Observations and time point of onset - Mean scores/animal - Reversibility	Reference
OECD 405 (2012), no deviations, study acceptable	Rabbit, New Zealand White, males, 3/group	24-epibrassinolide	0,1g/ eye, washout 24 hours after instillation	No effects observed Mean scores for all animals at all timepoints (cornea/ iris/ conjunctivae/ chemosis): 24 hours, animal 1: 0/ 0/ 0/ 0 48 hours, animal 2: 0/ 0/ 0/ 0 72 hours, animal 3: 0/ 0/ 0/ 0	[redacted] (2017)

2.6.2.5.1 Short summary and overall relevance of the provided information on serious eye damage/eye irritation

The acute eye irritation/corrosion study of 24-epibrassinolide (TGAI) in rabbits was investigated according to OECD test guideline 405. The test item, 0.1 g was placed in the conjunctival sac of the left eye of a single male rabbit. As no severe eye reactions were observed up to 72 hours approximately post treatment, the treatment was completed using the remaining two male rabbits.
The scoring of eye reactions was performed at 24, 48, 72 hours post test item instillation, the mean score was calculated across 3 scoring intervals (24, 48 and 72 hour post instillation).
The individual mean score for corneal opacity, iris effects, conjunctivae and chemosis for Animal No. 01, 02 and 03 was 0.00, 0.00, 0.00 and 0.00.

In Animal No. 01, 02 and 03, test item instillation in left eye of the rabbit caused slight redness of conjunctivae at 1 hour post test item instillation on day 0. The eye lesion was reversed at 24 hour observation and the treated eye appeared normal for all animals. The test item did not induce any corneal opacity, lesion in iris and chemosis throughout the experimental period. After 72 hour observation, the animals were sent for terminal sacrifice.
No clinical signs suggestive of systemic toxicity were observed in any of the animals throughout the acclimatization and post treatment periods.
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.
No gross pathological abnormalities were detected in any of the treated animals during necropsy at terminal sacrifice.

No reliable information on eye irritation was retrieved from public literature.

2.6.2.5.2 Comparison with the CLP criteria regarding serious eye damage/eye irritation

According to the criteria in CLP, this substance does not fulfil the criteria for classification as irritant to the eyes.

2.6.2.5.3 Conclusion on classification and labelling for serious eye damage/eye irritation

Conclusive and sufficient for classification. No classification proposed.

2.6.2.6 Respiratory sensitisation [equivalent to section 10.6 of the CLH report template]

Data lacking, no classification due to absence of data.

2.6.2.7 Skin sensitisation [equivalent to section 10.7 of the CLH report template]

Table 11: Summary table of animal studies on skin sensitisation

Method, guideline, deviations if any	Species, strain, sex, no/group	Test substance	Dose levels duration of exposure	Results	Reference
OECD 406, no deviations, study acceptable	Guinea pig, Albino, males, 10/group	24-epibrassinolide	Intradermal induction, epidermal application, and challenge	No reactions were observed after challenge exposure	[redacted] (2017)

2.6.2.7.1 Short summary and overall relevance of the provided information on skin sensitisation

For skin sensitisation, no effects were observed in the available guideline-compliant study.

No reliable information on sensitisation was retrieved from public literature.

2.6.2.7.2 Comparison with the CLP criteria regarding skin sensitisation

According to the criteria in CLP, this substance shall not be classified for skin sensitisation.

2.6.2.7.3 Conclusion on classification and labelling for skin sensitisation

Conclusive and sufficient for classification. No classification proposed.

2.6.2.8 Phototoxicity

Hazard class not applicable

2.6.2.9 Aspiration hazard [equivalent to section 10.13 of the CLH report template]

Hazard class is not applicable, the substance is a solid, and therefore the kinematic kinematic viscosity at 40°C is > 20.5 (see CLP-Regulation 3.10.3)

2.6.2.10 Specific target organ toxicity-single exposure (STOT SE) [equivalent to section 10.11 of the CLH report template]

Available relevant studies are summarized under sections B.2.6.2 (Acute Toxicity), B.2.6.6 (Developmental toxicity), and B2.6.7 (Neurotoxicity).

According to the "Guidance on the Application of the CLP Criteria", ECHA Version 5.0 July 2017, STOT-SE should be considered where there is clear evidence of toxicity to a specific organ, when it is observed in the absence of a classification for lethality.

According to CLP-criteria for classification as STOT-SE, associated guidance concentrations are as follows:

Category 1 (H370):
Substances that have produced significant toxicity in humans or that, on the basis of evidence from studies in experimental animals, can be presumed to have the potential to produce significant toxicity in humans following single exposure.
Substances are classified in Category 1 for specific target organ toxicity (single exposure) on the basis of:

a. reliable and good quality evidence from human cases or epidemiological studies; or

b. observations from appropriate studies in experimental animals in which significant and/or severe toxic effects of relevance to human health were produced at generally low exposure concentrations.

Guidance dose/concentration values are provided to be used as part of weight-of-evidence evaluation:

Rat (oral) ≤ 300 mg/kg bw
Rat or rabbit (dermal) ≤ 1000mg/kg bw
Rat (inhalation, dust/mist/fume): ≤ 1.0mg/l/4h

Category 2 (H371)
Substances that, on the basis of evidence from studies in experimental animals can be presumed to have the potential to be harmful to human health following single exposure
Substances are classified in Category 2 for specific target organ toxicity (single exposure) on the basis of observations from appropriate studies in experimental animals in which significant toxic effects, of relevance to human health, were produced at generally moderate exposure concentrations.

Guidance dose/concentration values are provided in order to help in classification.

Rat (oral) 2000 ≥ C > 300 mg/kg bw
Rat or rabbit (dermal) 2000 ≥ C > 1000mg/kg bw
Rat (inhalation, dust/mist/fume): 5.0 ≥ C > 1 mg/l/4h

Category 3 (H335/H336):
This category only includes narcotic effects and respiratory tract irritation. These are target organ effects for which a substance does not meet the criteria to be classified in Categories 1 or 2 indicated above. These are effects which adversely alter human function for a short duration after exposure and from which humans may recover in a reasonable period without leaving significant alteration of structure or function.

No significant non-lethal toxic effects were observed in the available studies. No indications for such effects were obtained in the reviewed literature. No indications for relevant effects in humans are available.

2.6.2.10.3 Conclusion on classification and labelling for STOT SE (specific target organ toxicity-single exposure)

Conclusive and sufficient for classification. No classification proposed.

2.6.3 Summary of repeated dose toxicity (short-term and long-term toxicity) [section 10.12 of the CLH report]

2.6.3.1 Specific target organ toxicity-repeated exposure (STOT RE) [equivalent to section 10.12 of the CLH report template]

Table 12: Summary table of animal studies on repeated dose toxicity (short-term and long-term toxicity) STOT RE (specific target organ toxicity - repeated exposure)

Method, guideline, deviations if any, species, strain, sex, no/group	**Test substance, route of exposure, dose levels, duration of exposure**	**Results - NOAEL/LOAEL - target tissue/organ - critical effects at the LOAEL**	**Reference**
Repeated dose 90 days oral toxicity study, OECD 408 (1998), Rat, Wistar 10 animals/group (main study) No deviations, study acceptable	24-epibrassinolide, Oral exposure, Preliminary study: 0, 500, 1000, 1500 mg/kg bw/day for 2 weeks Main study: 0, 100, 300, 1000 mg/ kg bw/day for 90 days	Preliminary study: Decreased food consumption at 1500 mg/kg bw/day Main study: Decreased food consumption Reduced body weight at 1000 mg/kg bw/day NOAEL = 300mg/kg bw/day	[redacted] (2017)

2.6.3.1.2 Comparison with the CLP criteria regarding STOT RE (specific target organ toxicity-repeated exposure)

The test item 24-Epibrassinolide (TGAI) was administrated daily by oral route to Wistar rats for a period of 90 consecutive days. A recovery period of 28 days without test item treatment was included in this study.

Three groups consisting of 10 male and 10 female rats each were treated at dose levels of 100, 300 and 1000 mg/kg bw/d respectively. Concurrently, vehicle control and test item high dose recovery groups (1000 mg/kg bw/d) were used in the present study.

All animals were observed for mortality/viability, detailed clinical signs and clinical signs of toxicity. Food consumption and body weight were recorded weekly during the treatment period. All animals were subject to Functional Observation Battery (FOB) examination during treatment period (week 13) and recovery (week 17). At the end of treatment and recovery periods all the animals were necropsied for gross and histopathology examinations.
All the animals survived up to the scheduled sacrifice.
No test item related changes in clinical signs, detailed clinical observation, ophthalmic examination findings, food consumption and body weight changes were noted.
Minor changes in hematological, clinical biochemistry, body weight, functional observation battery, body weight gain, organ weight and urine analysis were recorded at the end of treatment and recovery periods, but the variations were considered incidental as there was no dose dependent trend in the observed variations and the respective tissues revealed no abnormalities in macroscopic and histopathological examination.

Decreases in overall food consumption over the entire study period in both genders (not statistically significant), statistically significantly reduced body weights in male animals, and non-statistically significant body weight reductions in female animals were absorved at the highest dose level.

In conclusion, under the conditions of this experiment the No Observed adverse Effect Level (NOAEL) was determined to be 300 mg/kg body weight.

According to CLP-criteria for classification as STOT-RE, associated guidance concentrations are as follows:

Category 1 (H372):
Substances that have produced significant toxicity in humans or that, on the basis of evidence from studies in experimental animals, can be presumed to have the potential to produce significant toxicity in humans following repeated exposure.
Substances are classified in Category 1 for target organ toxicity (repeat exposure) on the basis of:
reliable and good quality evidence from human cases or epidemiological studies; or observations from appropriate studies in experimental animals in which significant and/or severe toxic effects, of relevance to human health, were produced at generally low exposure concentrations.

Equivalent guidance values for different study durations (oral only, since dermal and inhalative studies not relevant in this case):
Rat:
28-day: ≤ 30 mg/kg bw/d
90-day: ≤ 10 mg/kg bw/d

Category 2 (H373)
Substances that, on the basis of evidence from studies in experimental animals, can be presumed to have the potential to be Harmful to human health following repeated exposure.
Substances are classified in Category 2 for target organ toxicity (repeat exposure) on the basis of observations from appropriate studies in experimental animals in which significant toxic effects, of relevance to human health, were produced at generally moderate exposure concentrations.
Equivalent guidance values for different study durations (oral only, since dermal and inhalative studies not relevant in this case):
Rat:
28-day: ≤ 300 mg/kg bw/d
90-day: ≤ 100 mg/kg bw/d

No significant toxic effects were observed in the available guideline-compliant studies. No indications for significant non-lethal toxic effects were identified in the literature search, and no evidence from human cases is known.

2.6.3.1.3 Conclusion on classification and labelling for STOT RE (specific target organ toxicity-repeated exposure)

Conclusive and sufficient for classification. No classification proposed.

2.6.4 Summary of genotoxicity / germ cell mutagenicity [equivalent to section 10.8 of the CLH report template]

Table 13: Summary table of genotoxicity/germ cell mutagenicity tests *in vitro*

Method, guideline, deviations if any	Test substance	Relevant information about the study including rationale for dose selection (as applicable)	Observations /Results	Reference
Bacterial Reverse Mutation Test (AMES), OECD 471 (1997), no deviations, study acceptable	24-epibrassinolide	The assay was performed in two independent experiments both with and without liver microsomal activation. Each concentration, including the negative, vehicle and positive controls was tested in triplicates. The test item was tested at concentrations 0.0125, 0.0396, 0.1252, 0.3956 and 1.25 mg/plate, both in the presence (+S9) and absence (-S9) of metabolic activation.	No substantial increase in revertant colony numbers in any of the tester strains was observed following treatment with 24-Epibrassinolide (TGAI) at any dose level in both the confirmatory trials, neither in the presence nor in the absence of metabolic activation (S9 mix). There was also no tendency of higher mutation rates with increasing concentrations in the range below the generally acknowledged border of biological relevance. The solvent and positive controls induced the appropriate responses in the corresponding strains. It was concluded that 24-Epibrassinolide (TGAI) did not show a mutagenic potential in bacteria.	Srilatha S. (2017)
In vitro Mammalian Cell Gene Mutation Test (HPRT) OECD 476 (2015) No deviations, Study acceptable	24-epibrassinolide	The experiment was performed with a treatment time of 4 hours with and without metabolic activation. The maximum test item concentration of the pre-experiment (2000 µg/mL) was chosen with respect to the current OECD	No substantial and reproducible dose dependent increase of the mutation frequency was observed in the main experiment. Appropriate reference mutagens, used as positive controls, induced a distinct increase in mutant colonies and thus, showed the sensitivity of the test system and the activity of the metabolic activation system.	Wollny H.-E. (2017)

Method, guideline, deviations if any	Test substance	Relevant information about the study including rationale for dose selection (as applicable)	Observations /Results	Reference
		guideline 476. The concentration range of the main experiment was limited by cytotoxicity and precipitation of the test item.	In conclusion it can be stated that under the experimental conditions reported the test item did not induce gene mutations at the HPRT locus in V79 cells.	
In vitro Mammalian Chromosome Aberration Test, OECD 473 (2017) No deviations, study acceptable	24-epibrassinolide	The experiment was performed both in the presence and in the absence of metabolic activation (1 and 2% S9 mix) after 48 h mitogenic stimulation at concentrations of 0.063, 0.125, and 0.25 mg/mL. A moderate cytotoxic effect was observed after treatment with 0.25 mg/mL in both experimental parts. The reduction in the mitotic index was around 35% in the absence of S9 mix and 40% in the presence of S9 mix.	No relevant increase in cells carrying chromosomal aberrations was observed when compared to the values of the negative and solvent control and no evidence of an increase in polyploid metaphases was noticed after treatment with the test item as compared to the control cultures.	Kandula S.R. (2017)

2.6.4.1 Short summary and overall relevance of the provided information on genotoxicity / germ cell mutagenicity

The genotoxic potential of 24-Epibrassinolide was investigated in a number of different genotoxicity tests.
24-Epibrassinolide (TGAI) was negative in the bacterial reverse mutation test (Ames) and the mammalian cell gene mutation test (HPRT). In the chromosome aberration test, 24-Epibrassinolide (TGAI) showed no potential for clastogenicity with and without metabolic activation.
According to these findings, and due to ubiquitous lifetime exposure to 24-epibrassinloide, no *in vivo* studies were considered necessary by the applicant.
Open literature did not raise any concerns regarding possible genotoxic or mutagenic effects of 24-epibrassinolide.
No data on genotoxicity/ mutagenicity in humans is available.

2.6.4.2 Comparison with the CLP criteria regarding genotoxicity / germ cell mutagenicity

The following criteria for classification for germ cell mutagens are given in the CLP regulation:

The classification in Category 1A is based on positive evidence from human epidemiological studies. Substances to be regarded as if they induce heritable mutations in the germ cells of humans.

The classification in Category 1B is based on:
— positive result(s) from in vivo heritable germ cell mutagenicity tests in mammals; or
— positive result(s) from in vivo somatic cell mutagenicity tests in mammals, in combination with some evidence that the substance has potential to cause mutations to germ cells. It is possible to derive this supporting evidence from mutagenicity/genotoxicity tests in germ cells in vivo, or by demonstrating the ability of the substance or its metabolite(s) to interact with the genetic material of germ cells; or
— positive results from tests showing mutagenic effects in the germ cells of humans, without demonstration of transmission to progeny; for example, an increase in the frequency of aneuploidy in sperm cells of exposed people.

The classification in Category 2 is based on:
— positive evidence obtained from experiments in mammals and/or in some cases from in vitro experiments, obtained from:
— somatic cell mutagenicity tests in vivo, in mammals; or
— other in vivo somatic cell genotoxicity tests which are supported by positive results from in vitro mutagenicity assays.

Note: Substances which are positive in in vitro mammalian mutagenicity assays, and which also show chemical structure activity relationship to known germ cell mutagens, shall be considered for classification as Category 2 mutagens.

There is no evidence of mutagenicity/genotoxicity coming from epidemiological studies, no such studies are available.
No *in vivo* mutagenicity tests are available or are considered required by the applicant.
The available guideline-compliant *in vitro* tests do not indicate any potential for mutagenicity/ genotoxicity. There is no structure activity relationship to known germ cell mutagens.
In conclusion, the criteria for classification as germ cell mutagen are not met.

2.6.4.3 Conclusion on classification and labelling for genotoxicity / germ cell mutagenicity

Conclusive and sufficient for classification. No classification proposed.

2.6.5 Summary of long-term toxicity and carcinogenicity [equivalent to section 10.9 of the CLH report template]

No guideline-compliant studies on long term toxicity/ carcinogenicity were are available,.
Due to the ubiquitous presence of brassinosteroids in plant material and therefore continuous lifetime exposure via food and feed, conduct of long term toxicity/ carcinogenicity studies is not considered required by the applicant.
The literature review did not identify any studies addressing potential long term toxicity or carcinogenicity of 24-epibrassinolide. The publications submitted by the applicant did not raise any concerns regarding potential carcinogenic or other toxic effects after long term exposure.

2.6.5.1 Conclusion on classification and labelling for carcinogenicity

No classification proposed due to lack of data.

2.6.6 Summary of reproductive toxicity [equivalent to section 10.10 of the CLH report template]

No studies on reproductive toxicity were submitted or considered necessary by the applicant due to the ubiquitous presence of 24-epibrassinolide in food resulting in continuous lifetime exposure.
A study on developmental toxicity is available, no adverse effects were observed at the highest dose level tested (1000 mg/kg bw/day).
A literature study on homobrassinolide indicating no adverse effects up to 1000 mg/kg bw/day is also available.

2.6.6.1 Adverse effects on sexual function and fertility – generational studies [equivalent to section 10.10.1 of the CLH report template]

No data available.

2.6.6.1.2 Comparison with the CLP criteria regarding adverse effects on sexual function and fertility

Data lacking, no classification for effects on sexual function and fertility is proposed

2.6.6.2 Adverse effects on development [equivalent to section 10.10.4 of the CLH report template]

Table 14: Summary table of animal studies on adverse effects on development

Method, guideline, deviations if any, species, strain, sex, no/group	**Test substance, dose levels duration of exposure**	**Results** **- NOAEL/LOAEL (for parent, offspring and for developmental effects)** **- target tissue/organ** **- critical effects at the LOAEL**	**Reference**
Prenatal developmental toxicity study, OECD 414 (2001), Rat, Wistar,	24-epibrassinolide (Purity: 91.2% w/w) <u>Range finding study</u>: 0, 100, 300, 1000	<u>Range finding study:</u> No effects at 1000 mg/kg bw/day <u>Main study:</u> No effects up to the highest dose tested;	[redacted] (2017)

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, dose levels duration of exposure	Results - NOAEL/LOAEL (for parent, offspring and for developmental effects) - target tissue/organ - critical effects at the LOAEL	Reference
24 animals per dose group	mg/kg bw/day (gavage) Main Study: 0, 100, 300, 1000 mg/kg bw/day (gavage) Gestation days 5-19	NOAEL (maternal) 1000 mg/kg bw/d NOAEL (embryofœtal) 1000 mg/kg bw/d	

Table 15: Summary table of other studies relevant for developmental toxicity

Type of study/data	Test substance	Relevant information about the study (as applicable)	Observations	Reference
Prenatal developmental toxicity study, public literature	Homobrassinolide, trade name "Combine", purity 86,3%	Study design generally similar to OECD 414 (2001) Wistar rats Range finding study: 3 animals/group, no control group Main study: 20 animals per group Dosing from gestation day 6-15	Range finding study: 1000, 2000, 3000 mg/kg bw/day (gavage) ; severe toxicity at 2000 and 3000mg/kg bw/day Main study: 0, 100, 1000 mg/kg bw/day (gavage) No effects up to the highest dose tested; NOAEL (maternal) 1000 mg/kg bw/d NOAEL (embryofœtal) 1000 mg/kg bw/d	Murkunde and Murthy, (2010)

2.6.6.2.1 Short summary and overall relevance of the provided information on adverse effects on development

The test item, 24-epibrassinolide (TGAI), formulated in 0.1% sodium carboxymethyl cellulose was administered once daily by oral gavage to three treatment groups of twenty four pregnant female Wistar rats per group from gestation day 5 to day 19 at dose levels of 100, 300 and 1000 mg/kg bw/d. A control group of twenty four females was administered with vehicle (0.1% Sodium Carboxymethyl Cellulose) alone.

No clinical signs of illness were observed in any of the animals during treatment period. No test item related effects were observed in the body weight, body weight changes, corrected body weight gain and feed consumption.

The evaluation of the reproductive organs of the dam, the gravid uterine weight, ovarian weight, corpora lutea count, total implants, placental weight, pre and post implantation loss, litter size, viability of the foetus, foetus weight, foetal sex ratio, foetal anogenital distance and the external, visceral and skeletal examination of the foetuses, revealed that 24-epibrassinolide (TGAI) did not produce any maternal or embryofoetal toxicity at 100, 300 and 1000 mg/kg bw/day.

Hence, under the present experimental conditions, the No Observed Adverse Effect Level (NOAEL) of test item 24-epibrassinolide (TGAI) for maternal and embryofoetal toxicity in Wistar rats via oral route was found to be the highest dose level employed, i.e. 1000 mg/kg bw/day.

In a published study performed with homobrassinolide, no effects were observed up to 1000 mg/kg bw/day, which was the highest dose level tested in the main study.
In a range finding experiment, significant toxicity was observed at 2000 and 3000 mg/kg bw/day: Animals treated with 3000 mg/kg body weight/day of homobrassinolide were sacrificed humanely on GD 14 and 15 because of moribund condition (2/3). Rats treated with 2000 mg/kg body weight/day showed a very high decrease in body weight and feed consumption, and exhibited signs of toxicity like abortion (1/3), dullness, lethargy, and anorexia

during GD 8 to GD 17. Animals treated with 1000 mg/kg body weight exhibited dullness and lethargy during GD 8 to GD 10, which resolved subsequently. No further details on effects and their magnitude are available from this publication.

No human data are available.

Due to ubiquitous occurrence of 24-epibrassinolide and lifetime exposure via food, no further studies on reproductive toxicity were performed or are considered necessary by the applicant.

2.6.6.2.2 Comparison with the CLP criteria regarding adverse effects on development

Criteria for classification as reproductive toxicant according to CLP are as follows:

Category 1A:
Known human reproductive toxicant

Category 1B:
Presumed human reproductive toxicant largely based on data from animal studies
— clear evidence of an adverse effect on development in the absence of other toxic effects, or
— the adverse effect on reproduction is considered not to be a secondary non-specific consequence of other toxic effects

Category 2:
Suspected human reproductive toxicant
— some evidence from humans or experimental animals, possibly supplemented with other information, of an adverse effect on development and
— the evidence is not sufficiently convincing to place the substance in Category 1 (deficiencies in the study).
— the adverse effect on reproduction is considered not to be a secondary non-specific consequence of the other toxic effects

Since no data on humans is available, classification in category 1A is not applicable.

No adverse effects were observed in the available fully guideline compliant study on 24-epibrssinolide in Wistar rats up to the highest dose level tested (1000 mg/kg bw/day)

In a published study on homobrassinolide, a close analogue of 24-epibrassinolide, also no adverse effects were observed up to 1000mg/kg bw/day. However, significant effects were observed in a published study at 2000 and 3000 mg/kg bw/day.

Thus, since no adverse effects on reproduction were observed, classification is not proposed.

2.6.6.3 Adverse effects on or via lactation [equivalent to section 10.10.7 of the CLH report template]

2.6.6.3.2 Comparison with the CLP criteria regarding effects on or via lactation

Data lacking, no classification for effects via lactation is proposed

2.6.6.4 Conclusion on classification and labelling for reproductive toxicity

Adverse effects on sexual function and fertility: data lacking, no classification is proposed
Adverse effects on development: data conclusive, no classification is proposed
Adverse effects on or via lactation: data lacking, no classification is proposed

2.6.7 Summary of neurotoxicity

Through the dietary intake of plants and plant products, humans and animals are constantly exposed to brassinosteroids and their metabolites. Due to absence of neurotoxic effects from available guideline toxicity studies, and no indications from published literature for neurotoxic potential, no neurotoxicity studies are considered necessary.

2.6.8 Summary of other toxicological studies

2.6.8.1 Toxicity studies of metabolites and impurities

Table 16: Summary of QSAR studies submitted on 24-epibrassinolide and its impurities

Parameter investigated	Model	Result	Classification/ remarks	Reference
Similarity between 24-epibrassinolide and homobrassinolide	OECD-QSAR Toolbox (Version 3.4)	Read across between the two molecules is supported by this analysis, as both substances have the same profiles in the QSAR analysis	Ambiguous results for the endpoint 'Estrogen receptor binding', as contradicting results are obtained with two different predictive models	Wildemann T., 2015
Similarity between 24-epibrassinolide and impurities [redacted]	OECD-QSAR Toolbox (Version 3.4)	Comparing the impurities [redacted] to 24-Epibrassinolide no additional alert was found regarding the following endpoints: systemic toxicity, genotoxicity, carcinogenicity, endocrine disruption, reproductive and developmental toxicity and skin sensitisation. Impurity [redacted] met the inclusion criteria for skin irritation/corrosion, while 24-Epibrassinolide and the impurities [redacted] did not meet the criteria for inclusion.	No additional toxicological alerts compared to 24-epibrassinolide were predicted for impurities [redacted] except for a positive prediction on skin irritation/corrosion for Impurity [redacted]	Wildemann, T., Roth, T., 2015

2.6.8.2 Supplementary studies on the active substance

No studies raising concerns regarding potential adverse toxicological effects or effects relevant for classification were identified in in the literature search.

2.6.8.3 Endocrine disrupting properties

From the data available on 24-epibrassinolide, there are no indications that the active substance could act as endocrine disruptor.
A literature study performed with homobrassinolide including a Hershberger assay, was submitted with the dossier. As both substances are highly similar and, read across is considered justified. The authors conclude that oral application of homobrassinolide triggers a selective anabolic response with minimal or no androgenic side-effects.

2.6.9 Summary of medical data and information

New active substance, limited data available

2.6.10 Toxicological end points for risk assessment (reference values)

Table 17: Overview of relevant studies for derivation of reference values for risk assessment

Species	Study (method/type, length, route of exposure)	Test substance	Critical effect	NOAEL	LOAEL	Cross reference
Rat (Wistar)	90 days oral toxicity study	24-epibrassinloide	Reduced food consumption and body weight gain	300 mg/kg bw/day	1000 mg/kg bw/day	[redacted] (2017)
Rat (Wistar)	Developmental toxicity study	24-epibrassinoide	No effects up to the highest dose level tested	NOAEL (maternal) 1000 mg/kg bw/d NOAEL (embryofœtal) 1000 mg/kg bw/d	-	[redacted] (2017)

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. The available studies on toxicity demonstrated that 24-Epibrassinolide did not show any toxicological properties of concern. 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 were not conducted.
At the use rate and dilution applied for, exposure estimates for operators are in the ppm (µg/kg bw) range and thus considered lower than or comparable to naturally occurring, lifetime background exposure.

2.7 RESIDUE

2.7.1 Summary of storage stability of residues

Brassinosteroids, including 24-Epibrassinolide are naturally occurring plant constituents, present in higher plants, lower plants, including algae, mosses, the "living fossil" Equisetum as well as certain fungi. Brassinosteroids are present in all plant organs such as pollen, anthers, seeds, leaves, stems, roots, flowers, grains and fruits with the highest concentrations found in pollen, seeds and fruits. It is therefore impossible to distinguish between a possible residue resulting from the use of the natural-identical active substance 24-Epibrassinolide and the similar natural substance ubiquitously present in the environment.

The concentration of Brassinosteroids in plants is regulated by a complex system of feedback pathways and Brassinosteroids are constantly synthesised, metabolised, activated and inactivated depending on the plant's needs as well as environmental cues. The concentrations of Brassinosteroids are continuously fluctuating - spatially and temporally: in a single plant, different concentrations can be measured simultaneously in different plant organs, cell structures and cells as well as in the same location at different times. Different homeostatic mechanisms, including feedback inhibition of Brassinosteroids and catabolic inactivation, play a role in the maintenance of the equilibrium of bioactive Brassinosteroid in plants. Brassinosteroids exogenously applied to shoots and leafs are metabolised within 24-96 h after application.

Furthermore, 24-Epibrassinolide is an implicit candidate for inclusion in Annex IV of Regulation (EC) 396/2005 as it fulfils criteria 3 and 4 of SANCO/11188/2013 Rev. 2, 14 September 2015. Therefore, no more information on residues in EU is necessary.

Studies on the stability of residues were therefore not conducted.

2.7.2 Summary of metabolism, distribution and expression of residues in plants, poultry, lactating ruminants, pigs and fish

Brassinosteroids, including 24-Epibrassinolide, are naturally occurring plant constituents, found throughout the plant kingdom as well as some fungi. 24-Epibrassinolide elicits and activates the plant's self-defence mechanisms meditating the plant's resistance to unfavourable environmental factors, stress, and diseases.

They are present in higher plants, lower plants, including algae, mosses, the "living fossil" Equisetum and in certain fungi. Brassinosteroids are present in all plant organs such as pollen, anthers, seeds, leaves, stems, roots, flowers, grains and fruits with the highest concentrations found in pollen, seeds and fruits and considered an obligatory plant constituent. Pollen and immature seeds show the highest contents of Brassinosteroids with a range of 0.001 – 6400 µg/kg fresh weight, while shoots and leaves usually show lower concentrations of 0.001 – 100 µg/kg fresh weight.

The concentration of Brassinosteroids in plants is regulated by a complex system of feedback pathways and Brassinosteroids are constantly synthesised, metabolised, activated and inactivated depending on the plant's needs as well as environmental cues.

Thus, endogenous Brassinosteroid levels in plant tissues are primarily regulated through the tissue-specific control of Brassinosteroid synthesis, catabolism, and inactivation rather than through long-distance transport. Brassinosteriods produced in planta are only transported over short distances to the exterior surface of the producing cell or to the surfaces of neighboring cells.

Although Brassinosteroids are taken up through the roots and are translocated unchanged from roots to shoots through the plant's phloem, exogenously applied Brassinosteroids to shoots and leafs are not transported throughout the plant or only at a very low level.

The metabolic/catabolic pathway of 24-Epibrassinolide and Brassinosteroids in plants has not yet been completely elucidated. Nevertheless, some studies have been performed on the metabolic pathway in different plants and fungi. There is no uniform degradation pathway, but different plant and fungal species or group have their own pathways. As Brassinosteroids are phylogenetically ancient phytohormones, evolved in the Pre-Cambrian, it can be expected that each organism has developed its own co-evolutionary mechanism to metabolise these phytohormones.

These processes strongly depend on the plant species, the plant organ and the developmental stage. Different homeostatic mechanisms are thought to operate to maintain the Brassinosteroid equilibrium, including the feedback inhibition of Brassinosteroid production. The spatial and temporal regulation of its homeostasis at the tissue or at cellular level is crusial for normal growth and development of plants. In addition, catabolic inactivation is also considered to play a role in the regulation of bioactive Brassinosteroid levels.

Brassinosteroids are catabolically altered or conjugated, with some modifications yielding inactive products. Various processes like acylation, sulphonation, glycosylation hydroxylation etc. play a role in maintaining the optimum levels of the bioactive brassinosteroids in the cells.

Brassinosteroids exogenously applied to shoots and leafs are metabolised within 24-96 h after application.

In addition to that, for agricultural purposes, only very low amounts of the natural-identical synthesized molecule, 24-Epibrassinolide, are used and thus the artificial release will influence natural background levels only to a limited extend. For typical quantities of Brassinosteroids of 5-50 mg per hectare used in agriculture the authors calculate

for the highest dosage an average Brassinosteroids concentration of 2.1 x 10-3 nmol/g plant biomass assuming a total weight of 50 tons per hectare and full absorption by plants. The authors conclude that this is close to the natural Brassinosteroids concentration in plants. This is also compliant with the proposed application rates for the representative formulation.
As Brassinosteroids are phylogenetically ancient phytohormones, evolved in the Pre-Cambrian, it can be expected that each organism has developed its own co-evolutionary mechanism to metabolise these phytohormones. Animals such as poultry, lactating ruminants, pigs and fish are constantly exposed to 24-Epibrassinolide e.g. through consumption of plants as well as other natural foods. Furthermore, no different metabolic pathways of the natural-identical synthesized molecule, 24-Epibrassinolide, to the natural occurring 24-Epibrassinolide, are expected.

2.7.3 Definition of the residue

24-Epibrassinolide has no relevant toxicity hazard towards humans, mammals or animals, including aquatic organisms. Humans, mammals and fish are constantly exposed to 24-Epibrassinolide either directly through the consumption of primary material such as plants, plant organs (e.g. natural contents in seeds, roots, and leafs 0.22 - 378 µg/kg), natural foods such as honey (7.4 µg/kg) or algae (e.g. Bajguz A., 2009, please also refer to Vol. 3, B.7., Table 7.2.1-1) or indirectly e.g. through the consumption of herbivores.
EFSA has even concluded that plant sterols (which includes 24-Epibrassinolide) are not only of low risk for the human consumer but necessary for a healthy diet as they are contributing to lowering the LDL-cholesterol levels, which is pivotal for the prevention of coronary heart diseases. Therefore, a daily intake of up to 3 g of plant sterols per day is highly recommended by EFSA (see CA 5.9.2). This was also confirmed by studies performed on human volunteers where the effect of Brassinolide on cholesterol levels was evaluated (Statsenko *et al*., 2008).
Furthermore, Annex IV of Regulation (EC) 396/2005 of the European Parliament and of the Council on Maximum Residue Levels for pesticides contains a list of active substances for which maximum residue levels (MRLs) are not required. The criteria are listed in SANCO/11188/2013 Rev. 2, 14 September 2015: Guidance document on criteria for the inclusion of active substances into Annex IV of Regulation (EC) N° 396/2005.

Active substances of no toxicological concerns (criterion 3): This group consists of the active substances that fulfill all of the following criteria: ADI and ARfD are not needed, they are low risk substances [in the meaning of point 5 of Annex II of Regulation (EC) No 1107/2009 i.e., do not show any of the following properties: carcinogenic, mutagenic, toxic to reproduction, sensitising chemicals, very toxic or toxic, corrosive, endocrine disruptor, neurotoxic, immunotoxic] and they do not produce any adverse effect up to test guideline limit doses. In that case, no more information on residues in registered uses in EU is necessary.

Natural exposure is higher than the one linked to the use as PPP (criterion 4) substances which exhibit a higher natural background level in food than is expected from PPP use(s) (please see Vol. 3, B7., Table 7.2.1-1). Active substances for which exposure via usual diet is higher than the one through the use as PPP might also be eligible for the inclusion in Annex IV. This group also includes well-defined compounds, even if their toxicity is not negligible, if consumer exposure to the compound or its degradates via usual diet is higher than the one through use as PPP. In that case, no more information on residues in registered uses in EU is necessary.
24-Epibrassinolide has a very low toxicity profile and is ubiquitous distributed in the plant kingdom (please see Vol. 3, B7., Table 7.2.1-1) and therefore fulfils criterion 3 of SANCO/11188/2013 Rev. 2 of 14 September 2015: *The compound has no identified hazardous properties*. In addition, criterion 4 of SANCO/11188/2013 Rev. 2 of 14 September 2015: *Natural exposure is higher than the one linked to the use as PPP,* is met. Therefore, no more information on residues in registered uses in EU is necessary.
Therefore, 24-Epibrassinolde is considered a candidate for the inclusion in Annex IV of Regulation 396/2005.
No definition of residue is proposed for 24-Epibrassinolide.

2.7.4 Summary of residue trials in plants and identification of critical GAP

Brassinosteroids, including 24-Epibrassinolide are naturally occurring plant constituents found throughout the plant kingdom. They are present in higher plants, lower plants, including algae, mosses, the “living fossil” Equisetum as well as certain fungi. It is therefore impossible to distinguish between a possible residue resulting from the use of the natural-identical active substance 24-Epibrassinolide and the similar natural substance ubiquitously present in the environment.
Furthermore, very low concentrations of 24-Epibrassinolide (0.01 %) are intended to be applied as a spray application and residues only below the default MRL of 0.01 mg/kg or below the natural background level are expected.
Based upon the possible worst-case residue calculations for the representative uses, all crop residues are below the default MRL of 0.01 mg/kg except for wine and table grapes (0.01320 mg/kg). As assumption of no drift, 100 % crop interception by harvested crop, all applications conducted simultaneously at the day of harvest are a more than conservative approach, the residue levels are much lower for wine and table grapes in practice. Furthermore, based upon the theoretical application of 24-Epibrassinolide on rice, buckwheat, carrot, apple, tea and potatoes and

reference values for natural concentrations available (please refer to Table 7.3-2), all residue levels are below the default MRL of 0.01 mg/kg or below the measured natural background level (Table 7.3-2). In addition, no degradation or catabolism was assumed.

2.7.5 Summary of feeding studies in poultry, ruminants, pigs and fish

24-Epibrassinolide has no relevant toxicity hazard towards humans, mammals or animals, including aquatic organisms. Humans, mammals and fish are constantly exposed to 24-Epibrassinolide either directly through the consumption of primary material such as plants, plant organs (e.g. natural contents in seeds, roots, and leafs 0.22 - 378 µg/kg), natural foods such as honey (7.4 µg/kg) or algae or indirectly.
EFSA (2012) has even concluded that plant sterols (which includes 24-Epibrassinolide) are not only of low risk for the human consumer but necessary for a healthy diet as they are contributing to lowering the LDL-cholesterol levels, which is pivotal for the prevention of coronary heart diseases. Therefore, a daily intake of up to 3 g of plant sterols per day is highly recommended by EFSA (see CA 5.9.2). This was also confirmed in humans and rats, where the effect of Brassinolide on cholesterol levels was evaluated. In both, humans and rats, a decrease in total serum cholesterol from initial elevated values was observed.
Furthermore, 24-Epibrassinolide treatment of fingerlings of diverse fish species (e.g. black sea salmon, carp, Russian sturgeon and silver carp) lead to significantly less negative effects through toxicants (CuSO4, phenol) contained in the water and treatment of sturgeon eggs with 24-Epibrassinolide was found to increase fecundation, hatching and larvae/fingerling survival (higher resistance to stress). This effect was also found for phytophaguous fishes (grass carp and silver carp).
Due to the daily natural exposure of poultry, ruminants, pigs and fish to Brassinosteroids, feeding studies are not considered necessary.

2.7.6 Summary of effects of processing

24-Epibrassinolide has no relevant toxicity hazard towards humans, mammals or animals, including aquatic organisms. Humans, mammals and fish are constantly exposed to 24-Epibrassinolide either directly through the consumption of primary material such as plants, plant organs (e.g. natural contents in seeds, roots, and leafs 0.22 - 378 µg/kg), natural foods such as honey (7.4 µg/kg) or algae or indirectly.
EFSA (2012) has even concluded that plant sterols (which includes 24-Epibrassinolide) are not only of low risk for the human consumer but necessary for a healthy diet as they are contributing to lowering the LDL-cholesterol levels, which is pivotal for the prevention of coronary heart diseases. Therefore, a daily intake of up to 3 g of plant sterols per day is highly recommended by EFSA (see CA 5.9.2). This was also confirmed in humans and rats, where the effect of Brassinolide on cholesterol levels was evaluated. In both, humans and rats, a decrease in total serum cholesterol from initial elevated values was observed.
Furthermore, 24-Epibrassinolide treatment of fingerlings of diverse fish species (e.g. black sea salmon, carp, Russian sturgeon and silver carp) lead to significantly less negative effects through toxicants (CuSO4, phenol) contained in the water and treatment of sturgeon eggs with 24-Epibrassinolide was found to increase fecundation, hatching and larvae/fingerling survival (higher resistance to stress). This effect was also found for phytophaguous fishes (grass carp and silver carp).
Due to the daily natural exposure of poultry, ruminants, pigs and fish to Brassinosteroids, feeding studies are not considered necessary.

2.7.7 Summary of residues in rotational crops

Brassinosteroids, including 24-Epibrassinolide are naturally occurring plant constituents found throughout the plant kingdom. They are present in higher plants, lower plants, including algae and mosses, the “living fossil” Equisetum and in some fungi. Brassinosteroids are present in all plant organs such as pollens, anthers, seeds, leaves, stems, roots, flowers, grains and fruits with the highest concentrations found in pollen, seeds and fruits and considered an obligatory plant constituent.
Because Brassinosteroids are ubiquitous, phylogenetically ancient phytohormones that are naturally occurring throughout the plant kingdom, studies on the residues in rotational crops is not considered necessary.
Furthermore, 24-Epibrassinolide is a moderately hydrophobic organic compound with a log Pow of 2.0 (please refer to M CA 2) and will be spontaneously transferred from soil to the plant root by a diffusion-driven process. 24-Epibrassinolide is considered a “natural-identical synthesized molecule”, no differences in behaviour compared to the naturally available 24-Epibrassinolide are expected.

2.7.8 Summary of other studies

No further studies were conducted.

2.7.9 Estimation of the potential and actual exposure through diet and other sources

Acceptable Daily Intake (ADI) and Dietary Exposure Calculation

Humans are constantly exposed to 24-Epibrassinolide through consumption of plants and plant organs, e.g. seeds, roots, leafs and fruits (0.22 - 378 µg/kg), as well as other natural and processed foods such as honey (7.4 µg/kg), fruit juices (0.5 - 12 µg/kg) or wine (3 µg/kg) (Table 6.2.1-1).
EFSA (2012) has even concluded that plant sterols (which includes 24-Epibrassinolide) are not only of low risk for the human consumer but necessary for a healthy diet as they are contributing to lowering the LDL-cholesterol levels, which is pivotal for the prevention of coronary heart diseases. Therefore, a daily intake of up to 3 g of plant sterols per day is highly recommended by EFSA (see Vol. 3, B6.9 Medical data).
Furthermore, in the acute toxicity studies performed, 24-Epibrassinolide showed no effects at a concentration of 5000 mg/kg bw.
Based on the above justification, the establishment of an ADI is not considered necessary.

Acute Reference Dose (ARfD) and Dietary Exposure Calculation
Typically, an ARfD is based primarily on a NOAEL. As the ARfD is an oral limit value, it should therefore be derived from oral data. To translate the critical NOAEL into an ARfD, assessment factors accounting for uncertainties in extrapolation from toxicity data to the exposed human population have to be applied. These are based on a 10-fold factor for interspecies variability and a 10-fold factor for intra-individual variability when considering risks to the general population.
As no dose related effects were observed in acute toxicity studies, it is not possible to derive NOAELs. Therefore, no ARfD is proposed (Commission Directive 2008/113/EC).
As ARfD was not deemed necessary, acute risk assessment is not relevant.

2.7.10 Proposed MRLs and compliance with existing MRLs
As there is no residue definition, a proposal of MRLs for the representative crops is not relevant.

2.7.11 Proposed import tolerances and compliance with existing import tolerances
As there is no residue definition, a proposal of MRLs for the representative crops is not relevant.

2.8 FATE AND BEHAVIOUR IN THE ENVIRONMENT

2.8.1 Summary of fate and behaviour in soil

The degradation study from Chen *el al.* was not performed according the OECD guideline 307. Degradation rates were derived from the data available at 25 °C. RMS normalised the degradation rates to 20 °C. The degradation rate of the sterilized northeast China black soil was not taken into consideration in the calculation of the geometric mean. As only three values are available and not 4 as requested by the OECD guideline 307, and due to the flaws of the study, RMS considers the values to be of informative quality and the geometric mean not to be of sufficient strength to be used in modelling. The use of the worst case value of 69.55 days from the Jiangxi red soil was chosen by the RMS for further modelling. Though the DT_{50} in soil was estimated to be 69.55 days, explicit field studies are not considered to be of necessity for the assessment. This is based on the ubiquitous presence of brassinosteroids in the environment and the constant formation and decomposition of the substances.

No valid information is available for the determination of the mobility of 24-Epibrassionolide in soil. The available information provided indications on the mobility of the active substance within the different parts of plants, and also argumentation on the moderate hydrophobicity of the active substance to determine the mobility behaviour of the substance in soil. This information provides evidence of spontaneous transfer from soil or water to the plant by diffusion-driven process. This can partially be agreed upon, as the assumption that the active substance is therefore not available for leaching to the different water bodies or systems is not proven. Therefore, it is recommended to use the default K_{foc} of 0 L/kg as well as an opposite worst-case of 10000 L/kg.

2.8.2 Summary of fate and behaviour in water and sediment [equivalent to section 11.1 of the CLH report template]

The argumentation provided is mainly based on the presence of phytosteroids in soils, plants and fungi. The effects on cyanobacteria and on diatom are the only evidence of the influence of brassinosteroids in the aquatic compartment. Furthermore, absorption and metabolism of brassinosteroids were investigated in higher and lower plants, diatoms, green algae, fungi, mycobacteria, and cyanobacteria. Unfortunately, no rate of degradation in water-sediment systems could be determined from the open literature and the proposition that 24-Epibrassinolide is removed from water much faster than by hydrolysis alone could not be verified.

It is proposed to use the degradation value from the hydrolysis study at pH 7 and normalized to 20°C. This value of 31.48 days appears to describe the expected behaviour of 24-Epibrassinolide in water. It is estimated that the use of a default value of 1000 days would not be representative for 24-Epibrassinolide.

2.8.2.1 Rapid degradability of organic substances

Table 18: Summary of relevant information on rapid degradability

Method	Results*	Key or Supportive study	Remarks	Reference
No data				

** data on full mineralization should be reported*

2.8.2.1.1 Ready biodegradability

No specific study on ready biodegradability was available. Based on the hydrolytic degradation study, it cannot be fully agreed upon the fact that enough data is available to prove that >70 % of 24-Epibrassinolide would be metabolised within a 28-day period based on the half-life value for 24-Epibrassinolide of 24.1 days at pH 5 at 25 °C. The degradation rate k is 0.02876 d^{-1}. Following the equation for single first-order rate model (SFO) $C_t = C_0 e^{-kt}$ the reduction of the concentration of 24-Epibrassinolide after 28 days is calculated to be about 55 % rather than the requested 70 %. Therefore 24-epibrassinolide cannot be considered readily biodegradable.

2.8.2.1.2 BOD5/COD

No study was provided.

2.8.2.2 Other convincing scientific evidence

No further study was provided.

2.8.2.2.1 Aquatic simulation tests

No study was provided.

2.8.2.2.2 Field investigations and monitoring data (if relevant for C&L)

No study was provided.

2.8.2.2.3 Inherent and enhanced ready biodegradability tests

It cannot be fully agreed upon the fact that enough data is available to prove that >70 % of 24-epibrassinolide would be metabolised within a 28-day period based on the half-life value for 24-Epibrassinolide of 24.1 days at pH 5 at 25 °C. 24-epibrassinolide cannot be considered readily biodegradable, though it is a natural compound that is expected to degrade rapidly in the environment.

2.8.2.2.4 Soil and sediment degradation data

The degradation study from Chen el al. was not performed according the OECD guideline 307. Only three soils were investigated instead of the requested four soils. No biometer-type flasks or flow-through systems were used to monitor potential volatile products. Furthermore, there is no information on whether the flasks were incubated in the dark or in the presence of light. The recovery of brassinolide in soil was low to acceptable (84.3 – 93.2%).

Degradation rates were derived from the data available at 25 °C. RMS normalised the degradation rates to 20 °C and the rates are presented in the table below.

Type	Degradation rate at [d^{-1}] (25 °C)	Half-life [d] (25 °C)	Normalised DT_{50} [d] (20 °C)
Northeast China black soil	0.050	13.8	22.17
Sterilized Northeast China black soil	*0.049*	*14.1*	*22.65*
Henan fluvo-aquic soil	0.042	16.5	26.50
Jiangxi red soil	0.016	43.3	69.55
Geometric mean (n=3)		**21.44**	**34.44**

The degradation rate of the sterilized northeast China black soil (marked in italic in the table above) was not taken into consideration in the calculation of the geometric mean. As only three values are available, and due to the flaws of the study, RMS considers the values to be of informative quality and the geometric mean not to be of sufficient strength to be used in modelling. The use of the worst case value of 69.55 days from the Jiangxi red soil was chosen by the RMS for further modelling.

The DT_{50} in soil was estimated to be 69.55 days, though explicit field studies are not considered to be of necessity for the assessment. This is based on the ubiquitous presence of brassinosteroids in the environment and the constant formation and decomposition of the substances. No information on the degradation of 24-Epibrassinolide in sediment were available, but it is not expected to be different from the decomposition of natural 24-Epibrassinolide.

2.8.2.2.5 Hydrolysis

The study (Chen, S., Shi, L., Shan, Z., Hu, Q. (2005)) provides information on the hydrolysis of Brassinolide in three buffer solutions (pH 5, 7 and 9) at two temperatures (25 °C and 50 °C). The highest degradation rate is measured at a pH of 9 and a temperature of 50 °C. At 25 °C, Brassinolide has hydrolysis half-life values of 24.1 days at pH 5, 19.6 days at pH 7 and 16.4 days at pH 9. According to the authors, this can be considered as an easily hydrolysable substance. The notifier concluded that alkalinity and high temperature accelerated the hydrolysis of brassinolide, but the RMS could not identify significant proof to support this statement.

Temperature, °C	pH value	Degradation rate, d^{-1}	Half-life, d
25	5	0. 030	24. 1
	7	0. 036	19.6
	9	0. 042	16.4
50	5	0. 034	20. 9
	7	0. 043	16.3

	9	0. 051	13.6

RMS proposes to use the temperature normalised (for 20°C) value at pH 7 corresponding to 31.48 days for modelling of water-sediment systems (this value was calculated by the RMS). No degradation products were analysed during this study, but it is not expected that degradation products different than the ones obtained during the degradation of natural Brassinolides will be produced.

2.8.2.2.6 Photochemical degradation

Photolysis is not expected to contribute significantly to the degradation of 24-Epibrassinolide due to the low light absorbance of the active substance at a wavelength of 295 nm.

2.8.2.2.7 Other / Weight of evidence

None provided.

2.8.3 Summary of fate and behaviour in air

2.8.3.1 Hazardous to the ozone layer

Table 19: Summary table of studies on hazards to the ozone layer

Method	Results	Remarks	Reference
No data			

No specific information provided. No special experimental data are available on the active substance 24-Epibrassinolide or its products in air. However, 24-Epibrassinolide is naturally occurring and has a low volatility (vapour pressure: 1.90 x 10^{-15} Pa; calculated Henry's law constant 2.40 x 10^{-13} Pa·m^3/mol) and hence it is not considered to pose any significant concern in air.

2.8.3.1.1 Short summary and overall relevance of the provided information on hazards to the ozone layer

Not necessary.

2.8.3.1.2 Comparison with the CLP criteria

Not possible.

2.8.3.1.3 Conclusion on classification and labelling for hazardous to the ozone layer

Not possible.

2.8.4 Summary of monitoring data concerning fate and behaviour of the active substance, metabolites, degradation and reaction products

Not applicable.

2.8.5 Definition of the residues in the environment requiring further assessment

None.

2.8.6 Summary of exposure calculations and product assessment

Soil compartment

The assumptions made in Volume 3, CP 8.1.3 for the calculation of PEC_{SOIL} were based on worst-case application

and worst-case parameters, except for the use of a non-normalised DT_{50} value. This value was normalised and used in the calculations by the RMS. The temperature normalised DT_{50} was 69.55 days. The PEC_{SOIL} calculated were very low, in the range of 0.187 µg active substance/kg soil compared to mg/kg values with other substances. The initial PEC_{SOIL} is not much affected by the change of DT_{50}, but the PEC_{SOIL} at day 100 is almost doubled by the change. Though this change will have very little influence on the overall risk assessment, it is recommended for correctness. No plateau concentration was observed, hence it should be noted that due to the natural occurrence of phytobrassinolides, the substance 24-Epibrassinolide will be naturally found in soils at different concentrations, with sterol concentrations ranging from 0 up to 3600 mg/kg soil, depending on the measurements and the soils studied.

Groundwater compartment

No PEC_{GW} simulations were provided in Volume 3, CP 8.2.4, but an argumentation was presented based on the fact that no different leaching behaviour is to be expected between the natural occurring 24-Epibrassinolide and the synthesised 24-Epibrassinolide. Considering the fact that the PEC_{SOIL} for 24-Epibrassinolide is expected to be low, the argumentation is believed to provide enough evidence to predict that the potential of leaching of 24-Epibrassinolide into groundwater at concentrations influencing the natural concentrations of brassinosteroids will be low.

Water and sediment compartments

Step 1 and 2 simulations were performed for 24-Epibrassinolide in Volume 3, CP 8.2.5. The Rapporteur provided revised simulations based on normalised DT_{50} in soil and normalised hydrolysis DT_{50}. Furthermore, the Rapporteur provided a second set of simulation using a worst-case K_{fOC} value of 10000 L/kg to evaluate worst-case situations for the sediment compartment. Indeed, the use of a worst-case K_{fOC} value of 0 L/kg was only covering a worst-case situation for the water compartment only and the potential adsorption to sediment was underestimated. Applications on all crops were investigated. $PEC_{SW/SED}$ values for all crops are provided in Volume 3, CP 8.2.5 and are presented with the corresponding TER in section 2.9.9 of this document. No further risk mitigation measures were needed.

Air

No special experimental data are available on the active substance 24-Epibrassinolide or its products in air. However, 24-Epibrassinolide is naturally occurring and has a low volatility (vapour pressure: 1.90×10^{-15} Pa; calculated Henry's law constant 2.40×10^{-13} Pa·m^3/mol) and hence it is not considered to pose any significant concern in air.

Other routes of exposure of 24-Epibrassinolide to the environment can be excluded.

2.9 EFFECTS ON NON-TARGET SPECIES

2.9.1 Summary of effects on birds and other terrestrial vertebrates

In general the bridging from mammals to birds is considered acceptable for 24-Epibrassinolide since it is reasonable that the sterol metabolism does not differ drastically between these two vertebrate groups. However since no in-depth argumentation for the bridging was provided by the applicant as worst case assumption zRMS divided the mammalian endpoints by a factor of 10 for the risk assessment.

Table 20: Summary of 24-Epibrassinolide toxicity endpoints for birds

Test species	Test substance	Test design	End point	Toxicity	Reference
Rat, considered adequate for bridging to birds	24-Epibrassinolide (TGAI)	Acute, oral	LD_{50}	**> 500 mg a.s./kg bw/d[1]**	[redacted] (2017) KCA 8.1.2.1/0001
Rat, considered adequate for	Sunergist (0.01% 24-Epibrassinolide SL)	Acute, oral	LD_{50}	> 500 mg prod./kg bw/d[1] (equivalent to **> 0.05 mg a.s./kg bw/d**)	[redacted] (2017) KCP 7.1.1/0001

Test species	Test substance	Test design	End point	Toxicity	Reference
bridging to birds					
Rat, considered adequate for bridging to birds	24-Epibrassinolide (TGAI)	Long-term	NOEL	**= 100 mg a.s./kg bw/d**[1]	[redacted] (2017) KCA 5.6.2/0001

Bold values were used for the risk assessment.
[1] Endpoints of rat studies were used as surrogate for birds and divided by a factor of 10 for a worst case assumption

Table 21: Summary of 24-Epibrassinolide toxicity endpoints for mammals

Test species	Test substance	Test design	End point	Toxicity	Reference
Rat	24-Epibrassinolide (TGAI)	Acute, oral	LD_{50}	**> 5000 mg a.s./kg bw/d**	[redacted] (2017) KCA 8.1.2.1/0001
Rat	Sunergist (0.01% 24-Epibrassinolide SL)	Acute, oral	LD_{50}	> 5000 mg prod./kg bw/d (equivalent to **> 0.5 mg a.s./kg bw/d**)	[redacted] (2017) KCP 7.1.1/0001
Rat	24-Epibrassinolide (TGAI)	Long-term	NOEL	**= 1000 mg a.s./kg bw/d**	[redacted] (2017) KCA 5.6.2/0001

Bold values were used for the risk assessment.

2.9.2 Summary of effects on aquatic organisms [section 11.5 of the CLH report]

2.9.2.1 Bioaccumulation [equivalent to section 11.4 of the CLH report template]

Table 22: Summary of relevant information on bioaccumulation

Method	Species	Results	Key or Supportive study*	Remarks	Reference
Partition coefficient n-octanol/water according to OECD 117 & EEC A.8 [HPLC method]	-	log P_{OW} = 2.0	Key study	GLP study with 24-Epibrassinolide Batch No. 002-20150323 97.2% purity	Gao, J. (2015)
BCF: No data					

2.9.2.1.1 Estimated bioaccumulation

Due to the moderate lipophilic properties of 24-Epibrassinolide with a log Pow = 2.0 (i.e. below the cut-off value of log Pow ≥ 4 according to Regulation (EC) 1272/2008 under point 4.1.2.8.1) high potentials of bioaccumulation and bioconcentration are not to be expected. No experimentally determined BCF is available.

2.9.2.1.2 Measured partition coefficient and bioaccumulation test data

24-Epibrassinolide has a measured log Pow = 2.0 (Gao, 2015), i.e. below the cut-off value of log Pow ≥ 4 according to Regulation (EC) 1272/2008 under point 4.1.2.8.1. Therefore high potentials of bioaccumulation and bioconcentration are not to be expected. No experimentally determined BCF is available.

2.9.2.2 Acute aquatic hazard [equivalent to section 11.5 of the CLH report template]

Table 23: Summary of relevant information on acute aquatic toxicity

Method	Species	Test material	Results[1]	Key or Supportive study*	Remarks	Reference
Acute, 96 hr (static)	Zebrafish (*Danio rerio*)	24-Epibrassinolide	Mortality, LC_{50} > 5.0 (nom)	Key study	-	[redacted] (2017)
Acute, 48 hr (static)	*Daphnia magna*	24-Epibrassinolide	Mortality, EC50 > 2.86 (mm)	Key study	-	Matlock, D. & Moore, S. (2017)

2.9.2.2.1 Acute (short-term) toxicity to fish

One acute study was provided to assess the effects of the active substance 24-Epibrassinolide to fish (*Danio rerio*), the study is considered relevant and reliable with a toxicity endpoint of LC_{50} > 5.0 (nom). No study was submitted for the formulated product.

Table 24: Summary of acute toxicity endpoints for fish

Group	Test substance	Time scale (Test type)	Endpoint	Toxicity [mg a.s./L]	Reference
Fish					
Zebrafish (*Danio rerio*)	24-Epibrassinolide	Acute, 96 hr (static)	Mortality, LC_{50}	> 5.0 (nom)	[redacted] (2017)

2.9.2.2.2 Acute (short-term) toxicity to aquatic invertebrates

One acute study was provided to assess the effects of the active substance 24-Epibrassinolide to aquatic invertebrates (*Daphnia magna*), the study is considered relevant and reliable with a toxicity endpoint of LC_{50} > 2.86 (mm). No study was submitted for the formulated product.

Table 25: Summary of acute toxicity endpoints for invertebrates

Group	Test substance	Time scale (Test type)	Endpoint	Toxicity [mg a.s./L]	Reference
Aquatic invertebrates					
Daphnia magna	24-Epibrassinolide	Acute, 48 hr (static)	Mortality, EC_{50}	> 2.86 (mm)	Matlock, D. & Moore, S. (2017)

2.9.2.2.3 Acute (short-term) toxicity to algae or aquatic plants

No studies were submitted for the active substance or the formulated product for algae and macrophyts.

2.9.2.2.4 Acute (short-term) toxicity to other aquatic organisms

No additional acute studies were submitted for the active substance or the formulated product.

2.9.2.3 Long-term aquatic hazard [equivalent to section 11.6 of the CLH report template]

Table 26: Summary of relevant information on chronic aquatic toxicity

Method	Species	Test material	Results[1]	Key or Supportive study*	Remarks	Reference
No data						

2.9.2.3.1 Chronic toxicity to fish

No chronic studies were submitted for the active substance or the formulated product for fish.

2.9.2.3.2 Chronic toxicity to aquatic invertebrates

No chronic studies were submitted for the active substance or the formulated product for aquatic invertebrates.

2.9.2.3.3 Chronic toxicity to algae or aquatic plants

No studies were submitted for the active substance or the formulated product for algae and macrophyts.

2.9.2.3.4 Chronic toxicity to other aquatic organisms

No additional chronic studies were submitted for the active substance or the formulated product.

2.9.2.4 Comparison with the CLP criteria

2.9.2.4.1 Acute aquatic hazard

Table 27: Summary of information on acute aquatic toxicity relevant for classification

Method	Species	Test material	Results[1]	Remarks	Reference
Acute, 96 hr (static)	Zebrafish (*Danio rerio*)	24-Epibrassinolide	Mortality, LC_{50} > 5.0 (nom)	-	[redacted] (2017)
Acute, 48 hr (static)	*Daphnia magna*	24-Epibrassinolide	Mortality, EC50 > 2.86 (mm)	-	Matlock, D. & Moore, S. (2017)

No acute classification is triggered since all toxicity endpoints are > 1mg a.s./L. No formulation studies are available.

2.9.2.4.2 Long-term aquatic hazard (including bioaccumulation potential and degradation)

Table 28: Summary of information on long-term aquatic toxicity relevant for classification

Method	Species	Test material	Results[1]	Remarks	Reference
No log-term toxicity data available					

24-Epibrassinolide is characterized by a rather low solubility in water (3.8 mg/l, pH 4.8) at 20 °C. The active substance is considered as not rapidly degradable (please refer to the RMS fate comment in Vol. 3 CA B8 under point 7.2.2.1), the hydrolysis half-life is 31.48 days (temperature normalised for 20°C at pH 7), thus > 16 days (according to the CLP criteria trigger referenced in Regulation (EU) 1272/2008 and ECHA-17-G-21-EN). Further no details regarding the hydrolysis products are available.
No chronic toxicity studies are submitted. The effect studies of the active substance on algae and macrophyts were waived, however aquatic plants are considered to be potentially susceptible organisms, indicated by the public literature study Mekhalfi *et al.* (2012), due to possible growth stimulating effects in semi-static exposure laboratory conditions in tier 1 standard lab studies.

Overall potential effects on aquatic plants are considered unlikely but can't be fully excluded and therefore leave some grounds of concern leading to the proposal to classify 24-Epibrassinolide according to Regulation (EU) 1272/2008 within the "safety net" as hazard class "aquatic chronic cat. 4 (H413)" since no standard laboratory toxicity studies are presented for algae or macrophyts (i.e. no NOEC is available), the active substance has a rather low solubility in water and is not rapidly degradable.[1]

It is noted that the risk to aquatic plants is considered low when the representative formulation is applied according to the proposed GAP.

No study on degradability or other conclusive data is available, thus the active substance is considered as not rapidly degradable.

Due to the moderate lipophilic properties of 24-Epibrassinolide with a log Pow = 2.0 (i.e. below the cut-off value of log Pow ≥ 4 according to Regulation (EC) 1272/2008 under point 4.1.2.8.1) high potentials of bioaccumulation

[1] In the Regulation (EU) 286/2011 it stated regarding the classification of chronic cat. 4 that this includes "***for example***, *poorly soluble substances* […] *and which are not rapidly degradable* […] *and* [indicate] *a potential to bioaccumulate*". RMS understands the text in the regulation that the criteria listed in the regulation for a chronic cat. 4 classification are not exhaustive (referring to the phrase "for example"), i.e. a substance does not necessarily have to be poorly soluble AND not rapidly degradable AND bioaccumulative. Since 24-Epibrassinolide meets two of the listed criteria and due to the absence of standard lab studies for algae and macrophyts (i.e. no toxicity endpoints are available) some concern regarding potential effects on growth in semi-static tier 1 study settings remain. As no other scientific evidence is available to dismiss this concern the "safety net" classification aquatic chronic cat. 4 appears justifiable. It is noted, that both Regulation (EU) 286/2011 and ECHA-17-G-21-EN are not understood as exhaustive by RMS, since the phrase "for example" is used regarding the substance properties of a chronic cat. 4 classification.

and bioconcentration are not to be expected.

2.9.2.5 Conclusion on classification and labelling for environmental hazards

On the basis of the above information on acute and chronic toxicity, the following classification and labelling of 24-Epibrassinolide is proposed:

No Aquatic Acute classification: All acute toxicity endpoints are > 1 mg a.s./L

Aquatic Chronic 4 - H413 (May cause long lasting effects to aquatic life): Potential effects on growth of algae and macrophyts are considered unlikely but can't be fully excluded, leaving some grounds of concern since for algae and macrophyts no standard laboratory studies are presented (i.e. no toxicity endpoint available) and the active substance is considered as rather low soluble in water and not rapidly degradable.

2.9.3 Summary of effects on arthropods

Table 29: Summary of acute toxicity of 24-Epibrassinolide to honeybees

Species	Test substance	Time scale/type of endpoint	End point	Toxicity	Reference
Acute					
Apis mellifera	a.s., 24-Epibrassinolide	Acute (48 h)	Oral toxicity (LD_{50})	> 92.2 µg a.s./bee (actual consumed dose)	Bharathiraja, K. (2017a)
Apis mellifera	a.s., 24-Epibrassinolide	Acute (48 h)	Contact toxicity (LD_{50})	> 10 µg a.s./bee (solubility limit)	Bharathiraja, K. (2017b)
Chronic					
No chronic oral toxicity study with adult bees was submitted.					
Bee brood development					
No bee brood development study was submitted.					
Sub-lethal effects					
No sub-lethal effects study on bees (behavioural and reproductive) was submitted.					

Table 30: Summary of laboratory toxicity endpoints with standard sensitive non-target arthropod species

Species	Test substance	Time scale/type of endpoint	End point	Toxicity	Reference
Aphidius rhopalosiphi	Sunergist (0.01% 24-Epibrassinolide SL)	Acute, Tier I (glass plate exposure)	Mortality, LR_{50} Reproduction, ER_{50}	> 7000 mL product/ha (equivalent to > 0.69 g a.s./ha) > 7000 mL product/ha (equivalent to > 0.69 g a.s./ha)	Moll, M. (2017)
Typhlodromus pyri	Sunergist (0.01% 24-Epibrassinolide SL)	Acute, Tier I (glass plate exposure)	Mortality, LR_{50} Reproduction, ER_{50}	2831.9 mL product/ha (equivalent to 0.28 g a.s./ha) n.d.	Moll, M. (2017)
Additional species					
No additional studies submitted.					

n.d. … not determined

2.9.4 Summary of effects on non-target soil meso- and macrofauna

Table 31: Summary of effects on soil meso- and macrofauna

Test organism	Test substance	Application method of test a.s./ OM[1]	Time scale	End point	Toxicity
Earthworms					
	a.s.	-	Chronic	-	No data
Other soil macroorganisms					
	a.s.	-	-	-	No data

[1]To indicate whether the test substance was oversprayed/to indicate the organic content of the test soil (e.g. 5 % or 10 %).

2.9.5 Summary of effects on soil nitrogen transformation

No data.

2.9.6 Summary of effects on terrestrial non-target higher plants

No data.

2.9.7 Summary of effects on other terrestrial organisms (flora and fauna)

No other data concerning effects of the active substance 24-Epibrassinolide or the formulated product Sunergist to other terrestrial non-target organisms are available and are not a mandatory requirement.

2.9.8 Summary of effects on biological methods for sewage treatment

No effect studies were submitted by the notifier to address the effects on biological methods for sewage treatment. No data was presented by the applicant regarding natural background concentrations of 24-Epibrassinolide in sewage or sewage sludge. However taking into account that brassinosteroids naturally occur in plant matter and the environment the waiver is considered acceptable. Further it is reported in public literature study that microorganisms in general are capable to metabolise 24-Epibrassinolide. This provides together with low predicted environmental concentrations evidence that adverse effects to sewage sludge induced by 24-Epibrassinolide are considered unlikely. The data requirement was sufficiently addressed and no further risk assessment is necessary.

2.9.9 Summary of product exposure and risk assessment

2.9.9.1 Summary of product exposure and risk assessment for birds and mammals

The risk assessment for birds and mammals was conducted according to the EFSA Guidance Document on Risk Assessment for Birds and Mammals (EFSA Journal 2009;7(12):1438). For further details please refer to Vol. 3 CP B9 B.9.2.

Grapes (field use) at 3 x 0.05 g a.s./ha

Growth stage	Indicator or focal species	Time scale	DDD (mg/kg bw per day)	TER	Trigger
Active substance - Screening Step (Birds)					
All	Small omnivorous bird	Acute	0.00762	> 65617[1]	10
All	Small omnivorous bird	Long-term	0.00389	25707[1]	5
Formulated product - Screening Step (Birds)					
All	Small omnivorous bird	Acute	0.00762	**> 6.56[1]**	10
Formulated product – Tier 1 (Birds)					
BBCH 10 - 19	Small insectivorous bird "Redstart"	Acute	0.00219	> 23[1]	10
BBCH ≥ 20	Small insectivorous bird "Redstart"	Acute	0.00206	> 24[1]	10
BBCH 10 - 19	Small granivorous bird "Finch"	Acute	0.00118	> 42[1]	10
BBCH 20 - 39	Small granivorous bird "Finch"	Acute	0.00099	> 50[1]	10
BBCH ≥ 40	Small granivorous bird "Finch"	Acute	0.00059	> 84[1]	10
Ripening	Frugivorous bird "Thrush"	Acute	0.00231	> 22[1]	10
BBCH 10 - 19	Small omnivorous bird "lark"	Acute	0.00115	> 43[1]	10
BBCH 20 - 39	Small omnivorous bird "lark"	Acute	0.00096	> 52[1]	10
BBCH ≥ 40	Small omnivorous bird "lark"	Acute	0.00058	> 87[1]	10
Active substance - Screening Step (Mammals) – covering lettuce (Shortcut Value of grapes = SV of lettuce)					
All	Small herbivorous mammal	Acute	0.0109	> 458716	10
All	Small herbivorous mammal	Long-term	0.00723	138313	5
Formulated product - Screening Step (Mammals) – covering lettuce (Shortcut Value of grapes = SV of lettuce)					
All	Small herbivorous mammal	Acute	0.0109	> 46	10

Risk from bioaccumulation and food chain behaviour
Not relevant, as Log Pow is ≤ 3

Risk from consumption of contaminated water

Leaf scenario: not relevant for grapes

Puddle scenario, Screening step:

Not considered necessary, Koc is 0 (thus < 500 L/kg). The ratio between effective application rate and endpoint is below the trigger for less sorptive substances (< 50):

Birds, acute: 0.05/> 500[1] = < 0.0001

Birds, long-term: 0.05/100[1] = 0.0005

Mammals, acute: 0.05/> 5000 = < 0.00001

Mammals, long-term: 0.05/1000 = 0.00005

[1] Endpoints of rat studies were used as surrogate for birds and divided by a factor of 10 for a worst case assumption

Sugar beet (field use) at 3 x 0.04 g a.s./ha (also covering lettuce with 2 x 0.04 g a.s./ha)

Growth stage	Indicator or focal species	Time scale	DDD (mg/kg bw per day)	TER	Trigger
Active substance - Screening Step (Birds)					
All	Small omnivorous bird	Acute	0.01016	> 49213[1]	10
All	Small omnivorous bird	Long-term	0.00518	19305[1]	5
Formulated product - Screening Step (Birds)					
All	Small omnivorous bird	Acute	0.01016	**> 4.92[1]**	10
Formulated product – Tier 1 (Birds)					
Early (BBCH 10 – 19)	Small omnivorous bird "lark"	Acute	0.00154	> 33	10
Late (BBCH 30 – 49)	Small granivorous bird "Finch"	Acute	0.00158	> 32	10
BBCH 10 - 19	Small insectivorous bird "wagtail"	Acute	0.00070	> 72	10
BBCH 20 - 49	Small insectivorous bird "wagtail"	Acute	0.00049	> 101	10
BBCH 10 - 19	Small insectivorous bird "wagtail"	Acute	0.00070	> 72	10
BBCH 20 - 49	Small insectivorous bird "wagtail"	Acute	0.00161	> 31	10
Active substance - Screening Step (Mammals) – covered by grapes (Shortcut Value of grape > SV of sugar beet)					
All	Small herbivorous mammal	Acute	0.0109[2]	> 458716[2]	10
All	Small herbivorous mammal	Long-term	0.00723[2]	138313[2]	5
Formulated product - Screening Step (Mammals) – covered by grapes (Shortcut Value of grape > SV of sugar beet)					
All	Small herbivorous mammal	Acute	0.0109[2]	> 46[2]	10

Risk from bioaccumulation and food chain behaviour
Not relevant, as Log Pow is ≤ 3

Risk from consumption of contaminated water

Scenarios	Indicator or focal species	Time scale	PEC_{pool} x DWR	TER	Trigger
Leaf scenario (for lettuce)	Birds	Acute	0.0184	> 27174[1]	10

Puddle scenario, Screening step:
Covered by the assessment of grapes.

[1] Endpoints of rat studies were used as surrogate for birds and divided by a factor of 10 for a worst case assumption

[2]The risk assessment of grapes covers the assessment of sugar beet. The DDD of grape is considered worst case since it has a higher application rate the Shortcut Value of grapes is higher then the SV of lettuce

Cucurbits (glass house use) at 3 x 0.05 g a.s./ha

In uses in glass houses no exposure to birds and mammals is considered, therefore no risk assessment is necessary.

Summary of the risk assessment:

Birds:
The TER_A value of the active substance is above the trigger of 10 for acute exposure via application in grapes and sugar beet (covering all other uses), therefore an acceptable risk is demonstrated. However for the product Sunergist the acute TER is below 10, therefore a Tier 1 refinement is necessary. It is mentioned, that for the bird risk assessment the endpoints of rat studies were used as surrogate for birds and divided by a factor of 10 for a worst case assumption due to the absence of an in-depth bridging argumentation. Further 0% mortality occured in the acute rat toxicity study, thus this endpoint can be considered conservative.
The Tier 1 acute TER values are above the trigger of 10 for acute exposure via application in grapes and sugar beet (covering all other uses), therefore an acceptable risk to birds is demonstrated for the proposed use of the product Sunergist.

All TER_{Lt} values are above the trigger of 5 for long-term exposure in grapes, indicating an acceptable risk to birds from the use of the product.

As 24-Epibrassinolide has a log Pow of 2.0, no bioaccumulations is expected. Therefore, no risk assessment on food chain behaviour is considered necessary.
The $TER_{dw\ leaf}$ is above the trigger value of 10 for acute exposure, indicating an acceptable risk to birds via water consumed in leaf whorles.
The application rate/endpoint ratios are below the trigger value of 50 (for less sorptive substances with Koc < 500 L/kg) for acute and long-term exposure, thus no further assessment of the puddle scenario is necessary.

Mammals:
The TER_A value is above the trigger of 10 for acute exposure via application in grapes, therefore an acceptable risk is demonstrated. All other uses are covered by the risk assessment of grapes.

All TER_{Lt} values are above the trigger of 5 for long-term exposure in grapes, indicating an acceptable risk to mammals from the use of the product. All other uses are covered by the risk assessment of grapes.

As 24-Epibrassinolide has a log Pow of 2.0, no bioaccumulations is expected. Therefore, no risk assessment on food chain behaviour is considered necessary.
The application rate/endpoint ratios are below the trigger value of 50 (for less sorptive substances with Koc < 500 L/kg) for acute and long-term exposure, thus no further assessment of the puddle scenario is necessary.

2.9.9.2 Summary of product exposure and risk assessment for aquatic organisms

The risk assessment was carried out according to EFSA Journal 2013;11(7):3290. For further details please refer to Vol. 3 B9 CP B.9.4.

Table 32: PEC/RAC ratios for 24-Epibrassinolide for each organism group based on FOCUS calculations for the use of Sunergist in cucurbits (3 x 0.05 g a.s./ha), covering all other uses

Group		Fish acute	Inverteb. acute	Algae	Macrophyts
Test species		*Danio rerio*	*Daphnia magna*	No study submitted	No study submitted
Endpoint		LC_{50}	EC_{50}	E_rC_{50}	E_rC_{50}
(µg a.s./L)		> 5000	> 2860	No study submitted	No study submitted
AF		100	100	10	10
RAC (µg a.s./L)		50.00	28.60	-	-
FOCUS Scenario	**PEC $_{gl\text{-}max}$ (µg a.s./L)**				
Step 1 (cucurbits)					
	0.0514	< 0.0010	< 0.0018	-	-
Step 2 (cucurbits - north)					
	0.0232	< 0.0005	< 0.0008	-	-

The PEC/RAC ratio is below 1 for **fish** and **aquatic invertebrates**, indicating an acceptable acute risk to aquatic organisms following the proposed use of Sunergist based on active substance endpoints. Due to the low PEC/RAC ratios the large margin of safety is considered as acceptable to account for a potentially higher formulation toxicity compared to the effects of the active substance.
It is considered acceptable to waive the chronic fish and aquatic invertebrates studies due to the expected natural occurrence of brassinosteroids and other sterols in the environment and aquatic organisms. Further a low acute toxicity was demonstrated in fish and *Daphnia magna* and a generally low water solubility of 3.8 mg/L is reported for the active substance. Negative long-term effects to fish and aquatic invertebrates posed by 24-Epibrassinolide are therefore considered unlikely and an acceptable risk is concluded.

No studies were submitted by the notifier to address the effects on **algae**. No explicit data was presented by the applicant regarding natural background concentrations of 24-Epibrassinolide in surface water. In the study by Stirk et al. (2013) 24 microalgae species were analysed and the measured values of brassinolide ranged between 56.6 µg/g DW (Raphidocelis. subcapitata) and 548.7 µg/g DW (Klebsormidium flaccidum). Although 24-Epibrassinolide is not explicitly mentioned, the referenced brassinolide are considered to have a close structural relation or represent even precursors of 24-Epibrassinolide and are therefore seen suitable to serve as a proxy to support that algae plant tissue naturally contains the active substance in relevant amounts.
In the experiment by Mekhalfi et al. (2012) nominally 48 µg 24-Epibrassinolide/L were added to the growth medium of diatoms, but the study did neither follow an OECD test guideline nor was it performed according to GLP standards. No analytical verification is reported at all, therefore the actual exposure over time in this static test design remains unclear. However a worst case surface water PEC of 0.0232 µg a.s./L (FOCUS Step 2) was calculated in the fate section in Vol. 3 CP B 8. This worst case surface water concentration is > 2000 times below the concentration used in the study by Mekhalfi et al. (2012). Further in a public literature study by Hassett et al. (1977, reference KCA 8.2.5.4/0001, evaluated under point CA B.9.2.5.3) sterols were found in two North American lakes, with lake water sterol concentrations ranging from 0.7 – 3 µg/L. Due to their structural similarity the referenced sterols (cholesterol and β-sitosterol) are considered suitable to serve as a proxy to estimate the order of magnitude of 24-Epibrassinolide concentration in natural surface water.
This supports the assumption that the natural exposure to 24-Epibrassinolide can be considered to be higher than the exposure following an application of the active substance in the form of a plant protection product. Thus the argumentation to waive the standard laboratory study with algae is considered acceptable due to the expected natural occurrence of 24-Epibrassinolide and other brassinosteroids in the environment and aquatic organisms. Further a generally low water solubility of 3.8 mg/L (please refer to Vol. 3 CA Part B 2) is reported for the active substance. In conclusion adverse effects to algae posed by 24-Epibrassinolide and the application of the representative formulation Sunergist are considered unlikely and the data requirement was sufficiently addressed.

No studies were submitted by the notifier to address the effects on **aquatic macrophyts**. No explicit data was presented by the applicant regarding natural background concentrations of 24-Epibrassinolide in surface water. In public literature it is well documented, that brassinosteroids are considered essential for normal plant growth and development. Brassinosteroids, including 24-Epibrassinolide are naturally occurring, plant growth promoting molecules, present in higher plants and lower plants. Pollen and immature seeds contain the highest amount of brassinosteroids with a range of 1-100 µg/kg fresh weight, while shoots and leaves usually have lower amounts of 0.01 - 0.1 µg/kg fresh weight.

The study by Stirk et al. (2013; reference KCA 8.2.6/0002, evaluated under point CA B.9.2.6) shows that aquatic microalgae tissue of several species naturally contain brassinolide in relevant amounts (56.6 µg/g dry weight in Raphidocelis. subcapitata and 548.7 µg/g dry weight in Klebsormidium flaccidum). Stirk et al. (2014) found brassinolide in the seaweed tissue of E. maxima (4.58 – 12.50 ng/g dry weight). Therefore it is considered reasonable that also aquatic macrophyts naturally contain brassinosteroids (including 24-Epibrassinolide).
Further in a public literature study by Hassett et al. (1977, reference KCA 8.2.5.4/0001, evaluated under point CA B.9.2.5.3) sterols were found in two North American lakes, with lake water sterol concentrations ranging from 0.7 – 3 µg/L. Due to their structural similarity the referenced sterols (cholesterol and β-sitosterol) are considered suitable to serve as a proxy to estimate the order of magnitude of 24-Epibrassinolide concentration in natural surface water. A worst case surface water PEC of 0.0232 µg a.s./L (FOCUS Step 2) were calculated in the fate section in Vol. 3 CP B 8. This provides evidence that the natural exposure to 24-Epibrassinolide can be considered to be higher than the exposure following an application of the active substance in the form of a plant protection product. Thus the argumentation to waive the standard laboratory study with aquatic macrophyts is considered acceptable due to the expected natural occurrence of 24-Epibrassinolide and other brassinosteroids in the environment and aquatic organisms. Further a generally low water solubility of 3.8 mg/L (please refer to Vol. 3 CA Part B 2) is reported for the active substance. In conclusion adverse effects to aquatic macrophyts posed by 24-Epibrassinolide and the application of the representative formulation Sunergist are considered unlikely and the data requirement was sufficiently addressed.

2.9.9.3 Summary of product exposure and risk assessment of bees

The risk assessment for honey-bees was carried out both, according to the EFSA Bee Guidance Document (EFSA Journal 2013;11(7):3295) and according to the Terrestrial Ecotoxicology GD (SANCO/10329/2002) and is based on the application in grapes (3 x 0.05 g a.s./ha), which covers all other proposed uses. Since the new EFSA Bee GD (2013) is not yet noted by the Member States this risk assessment is only shown for informative purposes (i.e. not conclusive for authorization). For further details please refer to Vol. 3 CP B9 B.9.6.1.

Acute risk assessment:

Table 33: Risk assessment for 24-Epibrassinolide – use in grapes at 3 x 0.05 g a.s./ha (covering all other uses)

Terrestrial Ecotoxicology GD (SANCO/10329/2002)				
Species	Test substance	Risk quotient	HQ	Trigger
Apis mellifera	a.s., 24-Epibrassinolide	HQoral	< 0.0005	50
Apis mellifera	a.s., 24-Epibrassinolide	HQcontact	< 0.005	50
EFSA Bee GD (2013)				
Species	Test substance	Risk quotient	HQ/ETR	Trigger
Apis mellifera	a.s., 24-Epibrassinolide	HQcontact	< 0.005	85
Apis mellifera	a.s., 24-Epibrassinolide	ETRacute adult oral	< 0.000006	0.2

The oral hazard quotient (HQ_O) and the contact hazard quotient (HQ_C) are below the trigger of 50, indicating that the active substance 24-Epibrassinolide in the formulation poses a low risk to bees.

The $HQ_{contact}$ is below the trigger of 85, hence the acute contact toxicity to adult honey-bees is considered acceptable and no first tier risk assessment is required. Therefore, a low risk to bees is expected from application of Sunergist.

The $ETR_{acute\ oral\ (adult)}$ is below the trigger of 0.2, hence an acceptable acute oral risk is indicated. The use in grapes (3 x 0.05 g a.s./ha) covers all other intended uses. Therefore, a low risk to bees is expected from application of Sunergist.

Chronic risk consideration:

No studies were submitted to address chronic toxicity to adult bees posed by the active substance or the formulation, therefore no risk assessment can be performed. It is mentioned that the chronic risk assessment is not part of the currently noted risk assessment procedure.

There is evidence, that 24-Epibrassinolide is naturally found in various plants, pollen and honey. According to public literature, the highest concentration of 24-Epibrassinolide in bee pollen of *Vicia faba* was 5 µg/kg fresh weight and 7.4 ng 24-Epibrassinolide/g in honey.
Another public literature study assessed the effects of queen bees (Carnolian breed *Apis m. carnica*) fed with sugar syrup supplemented with phytohormones (including 0.12 mg epibrassinolide/L syrup). Queen bees fed 2 days before and for 2 days after insemination with the supplemented syrup did not show effects on egg laying behaviour or mortality. Although the study did not follow a standardized protocol and no explicit information regarding actual syrup uptake or housing conditions are presented the paper is still considered to support that negative effects posed by 24-Epibrassinolide are rather unlikely.

Assuming the maximum application rate of 0.05 g a.s./ha and the short cut value of 10.6, to achieve an acceptable risk (i.e. an ETR below the trigger of 0.03), the adult chronic oral toxicity endpoint (10-day LDD_{50}) has to be ≥ 0.018 µg a.s./bee/day.

Assuming the maximum application rate of 0.05 g a.s./ha and the short cut value of 6.1, to achieve an acceptable risk (i.e. an ETR below the trigger of 0.2), the chronic oral larvae toxicity endpoint (NOEL) has to be ≥ 0.002 µg a.s./larva/developmental period.

However no data was submitted to address whether or to which extent the application of Sunergist alters the 24-Epibrassinolide residue content in nectar and pollen. Thus a comparison with the value of 5 µg 24-Epibrassinolide/kg pollen referenced in the literature is not possible. Further it is noted that the non-target arthropod study with *Typhlodromus pyri* showed effects on reproduction at the lowest tested application rate (NOER < 0.048 g a.s./ha).

Therefore by weighting all this evidence, an unacceptable chronic risk is considered unlikely and negative chronic effects induced by 24-Epibrassinolide are also considered unlikely but appear to can't be fully excluded (since no data for the active substance or the formulation are available), especially for sensitive life stages. Member State views on this issue would be appreciated.

2.9.9.4 Summary of product exposure and risk assessment for non-target arthropods other than bees

The risk assessment was conducted according to the ESCORT 2 Guidance Document (2000) and is based on the application in grapes (3 x 0.05 g a.s./ha), which covers all other proposed uses. For further details please refer to Vol. 3 CP B9 B.9.6.2.

Table 34: First tier risk assessment for 24-Epibrassinolide - use in grapes at 3 x 0.05 g a.s./ha (covering all other uses)

Test substance	Species	Effect (LR_{50} g/ha)	HQ in-field	HQ off-field[1]	Trigger
Preparation, Sunergist (0.01% 24-Epibrassinolide SL)	*Aphidius rhopalosiphi*	> 0.69 g a.s./ha	< 0.17	< 0.06	2
Preparation, Sunergist (0.01% 24-Epibrassinolide SL)	*Typhlodromus pyri*	0.28 g a.s./ha	0.41	0.14	2

[1]drift factor 0.069, worst case use in grape

The in-field risk assessment indicates an acceptable risk to non-target arthropods other than bees following the application of Sunergist according to GAP, as the hazard quotient is below 2. No further risk assessment is necessary.

The off-field risk assessment indicates an acceptable risk to non-target arthropods other than bees following the application of Sunergist according to GAP, as the hazard quotient is below 2. No further risk assessment is necessary.

2.9.9.5 Summary of product exposure and risk assessment for soil meso- and macroorganisms

No risk assessment according to a guideline or guidance document is performed since no laboratory data is available. For further details please refer to Vol. 3 CP B9 B.9.8.1.

Qualitative risk consideration for soil organisms:

In public literature a natural occurrence in soil and a fast uptake of Brassinosteroids by plants via roots are reported. Further the degradation in soil (DT_{50} 69.02 d) and the low predicted environmental concentration in soil (PEC_{soil} = 0.2 µg/kg soil,) support that no adverse effects from the use of 24-Epibrassinolide and the representative formulation Sunergist (24-Epibrassinolide 0.01% SL) according to the proposed GAP are expected on soil organisms (earthworms and other soil macroorganisms). This maximum worst case PECsoil of 0.2 µg a.s./kg soil is around ~ 5000 times below the lowest reported soil sterol concentrations in public literature. Therefore an acceptable risk is concluded and no further risk assessment is required.

2.9.9.6 Summary of product exposure and risk assessment for soil nitrogen transformation rate

No risk assessment according to a guideline or guidance document is performed since no laboratory data is available. For further details please refer to Vol. 3 CP B9 B.9.10.

No studies were submitted to address the effects of the active substance or the formulated product on soil nitrogen transformation. However public literature and PECsoil calculations support that the natural exposure to 24-Epibrassinolide can be considered to be higher than the exposure following an application of the active substance in the form of a plant protection product. Therefore it is concluded that the risk to soil nitrogen transformation posed by the application of Sunergist (24-Epibrassinolide 0.01% SL) following the proposed GAP can be considered acceptable and no further risk assessment is required.

2.9.9.7 Summary of product exposure and risk assessment for non-target terrestrial plants

No risk assessment according to a guideline or guidance document is performed since no laboratory data is available. For further details please refer to Vol. 3 CP B9 B.9.12.

No laboratory studies were submitted to address the effects of the active substance on terrestrial non-target plants, but public literature studies support that brassinosteroids are commonly found in plant tissue and no negative effects are reported. It was reported that treatment of crop plants with brassinosteroids, including 24-Epibrassinolide increased crop health and yield.
Furthermore in efficacy trials with the product Sunergist no effects on phytotoxicity and vegetative vigour were found in grapes (with application rates up to 0.8 L product/ha in 600-800 L water), in lettuce (with application rates up to 0.8 L product/ha in 400 L water) and in sugar beet (with application rates up to 0.2 L product/ha in 200 L water).
Therefore it is concluded that waiving the effect studies on terrestrial non-target plants can be considered acceptable and negative effects on non-target plants induced by 24-Epibrassinolide and the representative formulation Sunergist (24-Epibrassinolide 0.01% SL) following the proposed GAP are considered unlikely.

2.9.9.8 Summary of product exposure and risk assessment for other terrestrial organisms (flora and fauna)

No risk assessment according to a guideline or guidance document is performed since no laboratory data is available. For further details please refer to Vol. 3 CP B9 B.9.14

No other data concerning effects of the active substance 24-Epibrassinolide or the formulated product Sunergist to other terrestrial non-target organisms are available and are not a mandatory requirement.

2.9.9.9 Summary of product exposure and risk assessment of effects on biological methods for sewage treatment

No risk assessment according to a guideline or guidance document is performed since no laboratory data is available. For further details please refer to Vol. 3 CA B9 B.9.8.

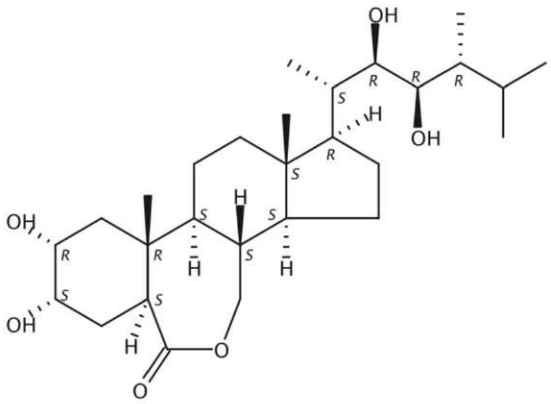
No effect studies were submitted by the notifier to address the effects on biological methods for sewage treatment. No data was presented by the applicant regarding natural background concentrations of 24-Epibrassinolide in sewage or sewage sludge. However taking into account that brassinosteroids naturally occur in plant matter and the environment the waiver is considered acceptable. Further it is reported in public literature study that microorganisms in general are capable to metabolise 24-Epibrassinolide. This provides together with low predicted environmental concentrations evidence that adverse effects to sewage sludge induced by 24-Epibrassinolide are considered unlikely. The data requirement was sufficiently addressed and no further risk assessment is necessary.

2.10 PROPOSED HARMONISED CLASSIFICATION AND LABELLING ACCORDING TO THE CLP CRITERIA *[SECTIONS 1-6 OF THE CLH REPORT]*

2.10.1 Identity of the substance [section 1 of the CLH report]

2.10.1.1 Name and other identifiers of the substance

Table 35: Substance identity and information related to molecular and structural formula of the substance

Name(s) in the IUPAC nomenclature or other international chemical name(s)	(3aS,5S,6R,7aR,7bS,9aS,10R,12aS,12bS)-10-[(2S,3R,4R,5R)-3,4-dihydroxy-5,6-dimethylheptan-2-yl]-5,6-dihydroxy-7a,9a-dimethylhexadecahydro-3H-benzo[c]indeno[5,4-e]oxepin-3-one
Other names (usual name, trade name, abbreviation)	24-Epibrassinolide
ISO common name (if available and appropriate)	-
EC number (if available and appropriate)	-
EC name (if available and appropriate)	-
CAS number (if available)	78821-43-9
Other identity code (if available)	-
Molecular formula	$C_{28}H_{48}O_6$
Structural formula	
SMILES notation (if available)	-
Molecular weight or molecular weight range	480.7 g/mol
Information on optical activity and typical ratio of (stereo) isomers (if applicable and appropriate)	Substance has enetiomeric centers. No S-stereoisomers are present in the active
Description of the manufacturing process and identity of the source (for UVCB substances only)	-
Degree of purity (%) (if relevant for the entry in Annex VI)	Min 900 g/kg

2.10.1.2 Composition of the substance

Table 36: Constituents (non-confidential information)

Constituent (Name and numerical identifier)	Concentration range (% w/w minimum and maximum in multi-constituent substances)	Current CLH in Annex VI Table 3.1 (CLP)	Current self-classification and labelling (CLP)
24-Epibrassinolide	90		

Table 37: Impurities (non-confidential information) if relevant for the classification of the substance

Impurity (Name and numerical identifier)	Concentration range (% w/w minimum and maximum)	Current CLH in Annex VI Table 3.1 (CLP)	Current self-classification and labelling (CLP)	The impurity contributes to the classification and labelling
none				

Table 38: Additives (non-confidential information) if relevant for the classification of the substance

Additive (Name and numerical identifier)	Function	Concentration range (% w/w minimum and maximum)	Current CLH in Annex VI Table 3.1 (CLP)	Current self-classification and labelling (CLP)	The additive contributes to the classification and labelling
none					

Table 39: Test substances (non-confidential information)

Identification of test substance	Purity	Impurities and additives (identity, %, classification if available)	Other information	The study(ies) in which the test substance is used
none				

2.10.2 Proposed harmonized classification and labelling

2.10.2.1 Proposed harmonised classification and labelling according to the CLP criteria

Table 40: Proposed harmonised classification and labelling according to the CLP criteria

	Index No	International Chemical Identification	EC No	CAS No	Classification		Labelling			Specific Conc. Limits, M-factors	Notes
					Hazard Class and Category Code(s)	Hazard statement Code(s)	Pictogram, Signal Word Code(s)	Hazard statement Code(s)	Suppl. Hazard statement Code(s)		
Current Annex VI entry											
Dossier submitters proposal	TBD	(3aS,5S,6R,7aR,7bS,9aS,10R,12aS,12bS)-10-[(2S,3R,4R,5R)-3,4-dihydroxy-5,6-dimethylheptan-2-yl]-5,6-dihydroxy-7a,9a-dimethylhexadecahydro-3H-benzo[c]indeno[5,4-e]oxepin-3-one; 24-epibrassinolide	N/A	78821-43-9	Aquatic Chronic 4	H413		H413			
Resulting Annex VI entry if agreed by RAC and COM	TBD	(3aS,5S,6R,7aR,7bS,9aS,10R,12aS,12bS)-10-[(2S,3R,4R,5R)-3,4-dihydroxy-5,6-dimethylheptan-2-yl]-5,6-dihydroxy-7a,9a-dimethylhexadeca	N/A	78821-43-9	Aquatic Chronic 4	H413		H413			

		hydro-3H-benzo[c]indeno[5,4-e]oxepin-3-one; 24-epibrassinolide									

2.10.2.2 Additional hazard statements / labelling

Table 41: Reason for not proposing harmonised classification and status under CLH public consultation

Hazard class	Reason for no classification	Within the scope of CLH public consultation
Explosives	Data conclusive but not sufficient for classification	Yes
Flammable gases (including chemically unstable gases)	Hazard class not applicable	No
Oxidising gases	Hazard class not applicable	No
Gases under pressure	Hazard class not applicable	No
Flammable liquids	Hazard class not applicable	No
Flammable solids	Data conclusive but not sufficient for classification	Yes
Self-reactive substances	Hazard class not applicable	No
Pyrophoric liquids	Hazard class not applicable	No
Pyrophoric solids	Data conclusive but not sufficient for classification	Yes
Self-heating substances	Data conclusive but not sufficient for classification	Yes
Substances which in contact with water emit flammable gases	Hazard class not applicable	No
Oxidising liquids	Hazard class not applicable	No
Oxidising solids	Data conclusive but not sufficient for classification	Yes
Organic peroxides	Hazard class not applicable	No
Corrosive to metals	Hazard class not applicable	No
Acute toxicity via oral route	Data conclusive but not sufficient for classification	Yes
Acute toxicity via dermal route	Data conclusive but not sufficient for classification	Yes
Acute toxicity via inhalation route	Data conclusive but not sufficient for classification	Yes
Skin corrosion/irritation	Data conclusive but not sufficient for classification	Yes
Serious eye damage/eye irritation	Data conclusive but not sufficient for classification	Yes
Respiratory sensitisation	Data lacking	No
Skin sensitisation	Data conclusive but not sufficient for classification	Yes
Germ cell mutagenicity	Data conclusive but not sufficient for classification	Yes
Carcinogenicity	Data conclusive but not sufficient for classification	Yes
Reproductive toxicity	Data conclusive but not sufficient for classification	Yes
Specific target organ toxicity-single exposure	Data conclusive but not sufficient for classification	Yes
Specific target organ toxicity-repeated exposure	Data conclusive but not sufficient for classification	Yes
Aspiration hazard	Hazard class not applicable	No

Hazard class	Reason for no classification	Within the scope of CLH public consultation
Hazardous to the aquatic environment	Hazard classification proposed	Yes
Hazardous to the ozone layer	Hazard class not assessed in this dossier	No

2.10.3 History of the previous classification and labelling

Not applicable, new active substance, no previous discussions

2.10.4 Identified uses

n.a.

2.10.5 Data sources

DAR sections B.6 (mammalian toxicology)

2.11 RELEVANCE OF METABOLITES IN GROUNDWATER

Not applicable, no metabolites identified

2.12 CONSIDERATION OF ISOMERIC COMPOSITION IN THE RISK ASSESSMENT

No isomers of 24-Epibrassinolide are present as relevant or significant impurities in the technical active substance. Therefore, isomeric compositions of 24-Epibrassinolide are not relevant for the risk assessments.

2.13 RESIDUE DEFINITIONS

2.13.1 Definition of residues for exposure/risk assessment

Food of plant origin: not required

Food of animal origin: not required

Soil: not required

Groundwater: not required

Surface water: not required

Sediment: not required

Air: not required

2.13.2 Definition of residues for monitoring

Food of plant origin: not required

Food of animal origin: not required

Soil: not required

Groundwater: not required

Surface water: not required

Sediment: not required

Air: not required

Level 3

24-EPIBRASSINOLIDE

3 PROPOSED DECISION WITH RESPECT TO THE APPLICATION

3.1 BACKGROUND TO THE PROPOSED DECISION

3.1.1 Proposal on acceptability against the decision making criteria – Article 4 and annex II of regulation (EC) No 1107/2009

3.1.1.1 Article 4				
		Yes	No	
i)	It is considered that Article 4 of Regulation (EC) No 1107/2009 is complied with. Specifically the RMS considers that authorisation in at least one Member State is expected to be possible for at least one plant protection product containing the active substance for at least one of the representative uses.	X		24-Epibrassinolide; Sunergist All uses according to GAP table
3.1.1.2 Submission of further information				
		Yes	No	
i)	It is considered that a complete dossier has been submitted	X		
ii)	It is considered that in the absence of a full dossier the active substance may be approved even though certain information is still to be submitted because: (a) the data requirements have been amended or refined after the submission of the dossier; or (b) the information is considered to be confirmatory in nature, as required to increase confidence in the decision.			
3.1.1.3 Restrictions on approval				
		Yes	No	
	It is considered that in line with Article 6 of Regulation (EC) No 1107/2009 approval should be subject to conditions and restrictions.		X	
3.1.1.4 Criteria for the approval of an active substance				
Dossier				
		Yes	No	
	It is considered the dossier contains the information needed to establish, where relevant, Acceptable Daily Intake (ADI), Acceptable Operator Exposure Level (AOEL) and Acute Reference Dose (ARfD).	X		Derivation of reference values is not required
	It is considered that the dossier contains the information necessary to carry out a risk assessment and for enforcement purposes (relevant for substances for which one or more representative uses includes use on	X		Brassinosteroids, including 24-Epibrassinolide, are naturally occurring plant growth promoting molecules. They are present in higher plants, lower plants,

	feed or food crops or leads indirectly to residues in food or feed). In particular it is considered that the dossier: (a) permits any residue of concern to be defined; (b) reliably predicts the residues in food and feed, including succeeding crops (c) reliably predicts, where relevant, the corresponding residue level reflecting the effects of processing and/or mixing; (d) permits a maximum residue level to be defined and to be determined by appropriate methods in general use for the commodity and, where appropriate, for products of animal origin where the commodity or parts of it is fed to animals; (e) permits, where relevant, concentration or dilution factors due to processing and/or mixing to be defined.			algae, mosses, the "living fossil" Equisetum as well as some fungi. Brassinosteroids are considered as an obligatory plant constituent. The intake of 24-Epibrassinolide through the use as a plant protection product will is similar to intake through average diet. This applies to the entire representative uses and the lead formulation submitted.
	It is considered that the dossier submitted is sufficient to permit, where relevant, an estimate of the fate and distribution of the active substance in the environment, and its impact on non-target species.	X		Applies to all uses according to GAP table
Efficacy				
		Yes	No	
	It is considered that it has been established for one or more representative uses that the plant protection product, consequent on application consistent with good plant protection practice and having regard to realistic conditions of use is sufficiently effective.	X		This applies to all uses except for the use as plant activator in cucurbits. However, since the number of GEP-trials is limited, further data are needed to confirm the claimed effects (elicitor - control of diseases; plant activator – antistress activity, increase of yield and quality of yield). Details see Level 2 resp. B.3 (PPP).
Relevance of metabolites				
		Yes	No	
	It is considered that the documentation submitted is sufficient to permit the establishment of the toxicological, ecotoxicological or environmental relevance of metabolites.	X		No relevant metabolites are identified
Composition				
		Yes	No	
	It is considered that the specification defines the minimum degree of purity, the identity and maximum content of impurities and, where relevant, of isomers/diastereo-isomers and additives, and the content of impurities of toxicological, ecotoxicological or environmental concern within acceptable limits.	X		*Min. purity 900 g/kg*

	It is considered that the specification is in compliance with the relevant Food and Agriculture Organisation specification, where such specification exists.	X		No FAO specification
	It is considered for reasons of protection of human or animal health or the environment, stricter specifications than that provided for by the FAO specification should be adopted			Not relevant
Methods of analysis				
		Yes	No	
	It is considered that the methods of analysis of the active substance, safener or synergist as manufactured and of determination of impurities of toxicological, ecotoxicological or environmental concern or which are present in quantities greater than 1 g/kg in the active substance, safener or synergist as manufactured, have been validated and shown to be sufficiently specific, correctly calibrated, accurate and precise.	X		*see 2.5*
	It is considered that the methods of residue analysis for the active substance and relevant metabolites in plant, animal and environmental matrices and drinking water, as appropriate, shall have been validated and shown to be sufficiently sensitive with respect to the levels of concern.			Not relevant
	It is confirmed that the evaluation has been carried out in accordance with the uniform principles for evaluation and authorisation of plant protection products referred to in Article 29(6) of Regulation 1107/2009.	X		
Impact on human health				
Impact on human health - ADI, AOEL, ARfD				
		Yes	No	
	It is confirmed that (where relevant) an ADI, AOEL and ARfD can be established with an appropriate safety margin of at least 100 taking into account the type and severity of effects and the vulnerability of specific groups of the population.	X		Due to the ubiquitous, lifetime exposure towards 24-epibrassinolide via food of plant origin and the absence of significant toxicological effects in the available guideline-compliant studies and scientific literature, derivation of reference values is not considered necessary.
Impact on human health – proposed genotoxicity classification				
		Yes	No	
	It is considered that, on the basis of assessment of higher tier genotoxicity testing carried out in accordance with the data requirements and other available data and information, including a review of the scientific literature, reviewed by the Authority, **the substance SHOULD BE classified or proposed for classification**, in accordance with the provisions of Regulation (EC) No 1272/2008, **as mutagen category 1A or 1B.**		X	24-Epibrassinolide was negative in the bacterial reverse mutation test (Ames) and the mammalian cell gene mutation test (HPRT). In the chromosome aberration test, 24-Epibrassinolide showed no potential for clastogenicity with and without metabolic activation. Open literature did not raise any concerns regarding possible genotoxic or mutagenic effects of 24-epibrassinolide. No data on genotoxicity/ mutagenicity in humans is available. According to these findings, and due to ubiquitous lifetime exposure to 24-epibrassinloide, no in vivo studies are considered necessary.

Impact on human health – proposed carcinogenicity classification				
		Yes	No	
i)	It is considered that, on the basis of assessment of the carcinogenicity testing carried out in accordance with the data requirements for the active substances, safener or synergist and other available data and information, including a review of the scientific literature, reviewed by the Authority, **the substance SHOULD BE classified or proposed for classification**, in accordance with the provisions of Regulation (EC) No 1272/2008, **as carcinogen category 1A or 1B.**		X	No guideline-compliant studies were submitted, due to the ubiquitous presence of brassinosteroids in plant material and therefore continuous lifetime exposure, conduct of long term toxicity/ carcinogenicity studies is not considered required. The literature review and the publications submitted by the applicant did not raise any concerns regarding potential carcinogenic or other toxic effects after long term exposure.
ii)	Linked to above classification proposal. It is considered that exposure of humans to the active substance, safener or synergist in a plant protection product, under realistic proposed conditions of use, is negligible, that is, the product is used in closed systems or in other conditions excluding contact with humans and where residues of the active substance, safener or synergist concerned on food and feed do not exceed the default value set in accordance with Article 18(1)(b) of Regulation (EC) No 396/2005.	X		Not applicable, no classification proposed
Impact on human health – proposed reproductive toxicity classification				
		Yes	No	
i)	It is considered that, on the basis of assessment of the reproductive toxicity testing carried out in accordance with the data requirements for the active substances, safeners or synergists and other available data and information, including a review of the scientific literature, reviewed by the Authority, **the substance SHOULD BE classified or proposed for classification**, in accordance with the provisions of Regulation (EC) No 1272/2008, **as toxic for reproduction category 1A or 1B.**		X	The oral administration of 24-epibrassinolide to pregnant Wistar rats by oral gavage from gestation day 5 to day 19 did not result in treatment related maternal and embryofoetal toxicity or mortalities. The NOAEL was found to be the highest dose level employed, i.e. 1000 mg/kg bw/day. In a study performed with homobrassinolide, no effects were observed up to 1000 mg/kg bw/day, which was the highest dose level tested in the main study. In a range finding experiment, significant toxicity was observed at 2000 and 3000 mg/kg bw/day. Due to ubiquitous occurrence of 24-epibrassinolide and lifetime exposure via food, no further studies on reproductive toxicity are considered necessary.
ii)	Linked to above classification proposal. It is considered that exposure of humans to the active substance, safener or synergist in a plant protection product, under realistic proposed conditions of use, is negligible, that is, the product is used in closed systems or in other conditions excluding contact with humans and where residues of the active substance, safener or synergist concerned on food	X		Not applicable, no classification proposed

	and feed do not exceed the default value set in accordance with Article 18(1)(b) of Regulation (EC) No 396/2005.			
Impact on human health – proposed endocrine disrupting properties classification				
		Yes	No	
i)	It is considered that **the substance SHOULD BE classified or proposed for classification** in accordance with the provisions of Regulation (EC) No 1272/2008, as **carcinogenic category 2 and toxic for reproduction category 2 and on that basis shall be considered to have endocrine disrupting properties**		X	Not applicable , no classification proposed
ii)	It is considered that **the substance SHOULD BE classified or proposed for classification** in accordance with the provisions of Regulation (EC) No 1272/2008, as **toxic for reproduction category 2 and** in addition the RMS considers the substance **has toxic effects on the endocrine organs and on that basis shall be considered to have endocrine disrupting properties**		X	No adverse effects related to endocrine activity of the substance were observed.
iii)	Linked to either i) or ii) immediately above. It is considered that exposure of humans to the active substance, safener or synergist in a plant protection product, under realistic proposed conditions of use, is negligible, that is, the product is used in closed systems or in other conditions excluding contact with humans and where residues of the active substance, safener or synergist concerned on food and feed do not exceed the default value set in accordance with Article 18(1)(b) of Regulation (EC) No 396/2005.	X		Not applicable, not considered to be an endocrine disruptor
Fate and behaviour in the environment				
Persistent organic pollutant (POP)				
		Yes	No	
	It is considered that the active substance **FULFILS** the criteria of a persistent organic pollutant (POP) as laid out in Regulation 1107/2009 Annex II Section 3.7.1.		X	The worst-case DT_{50} value in soil was estimated to be 69.55 days, though explicit field studies are not considered to be of necessity for the assessment.
Persistent, bioaccumulative and toxic substance (PBT)				
		Yes	No	
	It is considered that the active substance **FULFILS** the criteria of a persistent, bioaccumulative and toxic (PBT) substance as laid out in Regulation 1107/2009 Annex II Section 3.7.2.		X	The worst-case DT_{50} value in soil was estimated to be 69.55 days, though explicit field studies are not considered to be of necessity for the assessment.
Very persistent and very bioaccumulative substance (vPvB).				

		Yes	No	
	It is considered that the active substance **FULFILS** the criteria of a a very persistent and very bioaccumulative substance (vPvB) as laid out in Regulation 1107/2009 Annex II Section 3.7.3.		X	The worst-case DT_{50} value in soil was estimated to be 69.55 days, though explicit field studies are not considered to be of necessity for the assessment.
Ecotoxicology				
		Yes	No	
	It is considered that the risk assessment demonstrates risks to be acceptable in accordance with the criteria laid down in the uniform principles for evaluation and authorisation of plant protection products referred to in Article 29(6) under realistic proposed conditions of use of a plant protection product containing the active substance, safener or synergist. The RMS is content that the assessment takes into account the severity of effects, the uncertainty of the data, and the number of organism groups which the active substance, safener or synergist is expected to affect adversely by the intended use.	X		For all ecotoxicology sections an acceptable risk for all proposed uses is demonstrated, please refer to Level 2, 2.9.9 above for risk assessment summaries.
	It is considered that, on the basis of the assessment of Community or internationally agreed test guidelines, the substance **HAS** endocrine disrupting properties that may cause adverse effects on non-target organisms.		X	The available studies in the ecotoxicology section do indicate that 24-Epibrassinolide can be considered unlikely to exhibit endocrine disrupting properties. Please refer to Vol. 3 CA B9 under point B.9.1.5.
	Linked to the consideration of the endocrine properties immediately above. It is considered that the exposure of non-target organisms to the active substance in a plant protection product under realistic proposed conditions of use is negligible.	X		It is considered that the natural occurrence of 24-Epibrassinolide in terrestrial and aquatic environments is higher than the exposure following an application of the active substance in the form of a plant protection product. Please refer to Vol. 3 CP B9.
	It is considered that it is established following an appropriate risk assessment on the basis of Community or internationally agreed test guidelines, that the use under the proposed conditions of use of plant protection products containing this active substance, safener or synergist: — will result in a negligible exposure of honeybees, or — has no unacceptable acute or chronic effects on colony survival and development, taking into account effects on honeybee larvae and honeybee behaviour.	X		An acceptable acute risk is demonstrated for all proposed uses, please refer to Level 2, 2.9 for risk assessment summaries. Negative effects on bees following an exposure to 24-Epibrassinolide are considered unlikely. By weighting all evidence, an unacceptable chronic risk is considered unlikely and negative chronic effects induced by 24-Epibrassinolide are also considered unlikely but appear to can't be fully excluded (since no data for the active substance or the formulation are available), especially for sensitive life stages. Member State views on this issue would be appreciated. Please refer to Level 2, 2.9.9 above and Vol. 3 CP B9 B.9.6.1.
Residue definition				

		Yes	No	
	It is considered that, where relevant, a residue definition can be established for the purposes of risk assessment and for enforcement purposes.	X		Not required
Fate and behaviour concerning groundwater				
		Yes	No	
	It is considered that it has been established for one or more representative uses, that consequently after application of the plant protection product consistent with realistic conditions on use, the predicted concentration of the active substance or of metabolites, degradation or reaction products in groundwater complies with the respective criteria of the uniform principles for evaluation and authorisation of plant protection products referred to in Article 29(6) of Regulation 1107/2009.	X		It is not expected that the application of synthetic 24-Epibrassinolide will influence the natural concentrations of phytosteroids or brassinolides, and will most probably not be present in groundwater at concentrations above 0.1 µg/L.

3.1.2 Proposal – Candidate for substitution

Candidate for substitution				
		Yes	No	
	It is considered that the active substance shall be approved as a candidate for substitution		X	

3.1.3 Proposal – Low risk active substance

Low-risk active substances				
		Yes	No	
	It is considered that the active substance **shall be considered of low risk.** In particular it is considered that the substance **should NOT be classified or proposed for classification** in accordance with Regulation (EC) No 1272/2008 as at least one of the following: — carcinogenic, — mutagenic, — toxic to reproduction, — sensitising chemicals, — very toxic or toxic, — explosive, — corrosive. In addition it is considered that **the substance is NOT**: — persistent (half-life in soil more than 60 days), — has a bioconcentration factor higher than 100, — is deemed to be an endocrine disrupter, or — has neurotoxic or immunotoxic effects.	X		24-epibrassinolide is considered to be of low risk: The substance **is NOT be classified or proposed for classification** in accordance with Regulation (EC) No 1272/2008 as at least one of the following: — carcinogenic: No — mutagenic No — toxic to reproduction: No — sensitising chemicals: No — very toxic or toxic: No — explosive: No — corrosive: No In addition it is considered that **the substance is NOT**: — persistent (half-life in soil more than 60 days): No — has a bioconcentration factor higher than 100: No — is deemed to be an endocrine disrupter, No — has neurotoxic or immunotoxic effects: No

3.1.4 List of studies to be generated, still ongoing or available but not peer reviewed

Data gap	Relevance in relation to representative use(s)	Study status		
		No confirmation that study available or on-going.	Study on-going and anticipated date of completion	Study available but not peer-reviewed
3.1.4.1 Identity of the active substance or formulation				
none				
3.1.4.2 Physical and chemical properties of the active substance and physical, chemical and technical properties of the formulation				
none				
3.1.4.3 Data on uses and efficacy				
none				
3.1.4.4 Data on handling, storage, transport, packaging and labelling				
none				
3.1.4.5 Methods of analysis				
none				

3.1.4.6 Toxicology and metabolism				
none				
3.1.4.7 Residue data				
none				
3.1.4.8 Environmental fate and behaviour				
none				
3.1.4.9 Ecotoxicology				
none				

3.1.5 Issues that could not be finalised

An issue is listed as an issue that could not be finalised where there is not enough information available to perform an assessment, even at the lowest tier level, for the representative uses in line with the Uniform Principles, as laid out in Commission Regulation (EU) No 546/2011, and where the issue is of such importance that it could, when finalised, become a concern (which would also be listed as a critical area of concern if it is of relevance to all representative uses).

Area of the risk assessment that could not be finalised on the basis of the available data	**Relevance in relation to representative use(s)**
	[specify if measure relates to a specific representative use/use scenario/product or to all uses/products]

3.1.6 Critical areas of concern

An issue is listed as a critical area of concern:
(a) where the substance does not satisfy the criteria set out in points 3.6.3, 3.6.4, 3.6.5 or 3.8.2 of Annex II of Regulation (EC) No 1107/2009 and the applicant has not provided detailed evidence that the active substance is necessary to control a serious danger to plant health which cannot be contained by other available means including non-chemical methods, taking into account risk mitigation measures to ensure that exposure of humans and the environment is minimised, or
(b) where there is enough information available to perform an assessment for the representative uses in line with the Uniform Principles, as laid out in Commission Regulation (EU) 546/2011, and where this assessment does not permit to conclude that for at least one of the representative uses it may be expected that a plant protection product containing the active substance will not have any harmful effect on human or animal health or on groundwater or any unacceptable influence on the environment.

An issue is also listed as a critical area of concern where the assessment at a higher tier level could not be finalised due to a lack of information, and where the assessment performed at the lower tier level does not permit to conclude that for at least one of the representative uses it may be expected that a plant protection product containing the active substance will not have any harmful effect on human or animal health or on groundwater or any unacceptable influence on the environment.

Critical area of concern identified	**Relevance in relation to representative use(s)**
	[specify if concern relates to all or specific representative use/use scenario/product or to all uses/products]

3.1.7 Overview table of the concerns identified for each representative use considered

(If a particular condition proposed to be taken into account to manage an identified risk, as listed in 3.3.1, has been evaluated as being effective, then 'risk identified' is not indicated in this table.)
All columns are grey as the material tested in the toxicological studies has not been demonstrated to be representative of the technical specification.

Representative use		All uses (X[1])
Operator risk	Risk identified	
	Assessment not finalised	
Worker risk	Risk identified	
	Assessment not finalised	
Bystander risk	Risk identified	
	Assessment not finalised	
Consumer risk	Risk identified	
	Assessment not finalised	
Risk to wild non target terrestrial vertebrates	Risk identified	
	Assessment not finalised	
Risk to wild non target terrestrial organisms other than vertebrates	Risk identified	
	Assessment not finalised	
Risk to aquatic organisms	Risk identified	
	Assessment not finalised	
Groundwater exposure active substance	Legal parametric value breached	
	Assessment not finalised	
Groundwater exposure metabolites	Legal parametric value breached	
	Parametric value of 10µg/L[(a)] breached	
	Assessment not finalised	
Comments/Remarks		

The superscript numbers in this table relate to the numbered points indicated within chapter 3.1.5 and 3.1.6. Where there is no superscript number, see level 2 for more explanation.
(a): Value for non relevant metabolites prescribed in SANCO/221/2000-rev 10-final, European Commission, 2003

3.1.8 Area(s) where expert consultation is considered necessary

It is recommended to organise a consultation of experts on the following parts of the assessment report:

Area(s) where expert consultation is considered necessary	Justification

3.1.9 Critical issues on which the Co RMS did not agree with the assessment by the RMS

n.a.

3.2 PROPOSED DECISION

[redacted]

3.3 RATIONAL FOR THE CONDITIONS AND RESTRICTIONS TO BE ASSOCIATED WITH THE APPROVAL OR AUTHORISATION(S), AS APPROPRIATE

[redacted]

3.3.1 Particular conditions proposed to be taken into account to manage the risks identified

Proposed condition/risk mitigation measure	Relevance in relation to representative use(s)
[redacted]	[redacted]

3.4 APPENDICES

GUIDANCE DOCUMENTS USED IN THIS ASSESSEMENT

General

Section identity, physical chemical and analytical methods

Section physico chemical properties
none

Section analytical methods
Guidance for generating and reporting methods of analysis in support of pre-registration data requirements for Annex II (part A, Section 4) and Annex III (part A, Section 5) of Directive 91/414 *(SANCO/3029/99 rev. 4)*
Guidance for generating and reporting methods of analysis in support of pre- and post-registration data requirements for Annex II (part A, Section 4) and Annex III (part A, Section 5) of Directive 91/414 *(SANCO/3030/99 rev. 4)*
Guidance document on pesticide residue analytical methods *(SANCO/825/00 rev. 8.1)*

Section Data on application and efficacy

Guidance document for applicants on preparing dossiers for the approval of a chemical new active substance and for the renewal of approval of a chemical active substance according to Regulation (EU) 283/2013 and Regulation (EU) No 284/2013 (SANCO/10181/2013– rev. 3, December 2014).
Guidance document on data requirements on efficacy for the dossier to be submitted for the approval of new active substances contained in plant protection products (SANCO/10054/2013-rev.3 of 11. July 2013).

Section Toxicology

EFSA (2011). Submission of scientific peer-reviewed open literature for the approval of pesticide active substances under Regulation (EC) No 1107/2009 (OJ L 309, 24.11.2009, p. 1-50). EFSA Journal 2011;9(2):2092. [49 pp.]. doi:10.2903/j.efsa.2011.2092

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

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

ECHA : Guidance on the Application of the CLP Criteria, Version 5.0 (July 2017)

Guidance document for applicants on preparing dossiers for the approval of a chemical new active substance and for the renewal of approval of a chemical active substance according to Regulation (EU) 283/2013 and Regulation (EU) No 284/2013 (SANCO/10181/2013– rev. 2, May 2013)

Section Residue and consumer risk assessment

none

Section fate and behavior in environment

EFSA (2011). Submission of scientific peer-reviewed open literature for the approval of pesticide active substances under Regulation (EC) No 1107/2009 (OJ L 309, 24.11.2009, p. 1-50). EFSA Journal 2011;9(2):2092. [49 pp.]. doi:10.2903/j.efsa.2011.2092.
EFSA (2014). EFSA Guidance Document for evaluating laboratory and field dissipation studies to obtain DegT50 values of active substances of plant protection products and transformation products of these active subsrances in soil. EFSA Journal 2014; 12(5):3662, 37pp.
EC (2014) Generic guidance for Estimating Persistence and Degradation Kinetics from Environmental Fate Studies on Pesticides in EU Registration, version: 1.1, 18 December 2014
EC (2014) Assessing Potential for Movement of Active Substances and their Metabolites to Ground Water in the

EU. Sanco/13144/2010, version 3, 10 October 2014
EC (2015) Generic guidance for FOCUS surface water Scenarios. Version: 1.4, May 2015
FOCUS (2007). Landscape And Mitigation Factors In Aquatic Risk Assessment. Volume 1. Extended Summary and Recommendations. Report of the FOCUS Working Group on Landscape and Mitigation Factors in Ecological Risk Assessment, EC Document Reference SANCO/10422/2005 v2.0. 169 pp.
FOCUS (2008) Pesticides in air: Considerations for exposure assessment. SANCO/10553/2006, Rev 2, June 2008

Section ecotoxicology

EFSA (European Food Safety Authority), 2009. Guidance Document on Risk Assessment for Birds and Mammals on request of EFSA. EFSA Journal 2009; 7(12):1438.

EFSA (European Food Safety Authority), 2013. EFSA Scientific Opinion. Guidance on tiered risk assessment for plant protection products for aquatic organisms in edge-of-field surface waters. EFSA Journal 2013; 11(7): 3290.

EFSA (European Food Safety Authority), 2013. EFSA Guidance Document on the risk assessment of plant protection products on bees (*Apis mellifera*, *Bombus* spp. and solitary bees). EFSA Journal 2013;11(7):3295.

SANCO/10329/2002 rev.2. Guidance Document on Terrestrial Ecotoxicology Under Council Directive 91/414/EEC. SANCO/10329/2002 rev.2 final, 17 October 2002.

ESCORT 2: Candolfi, M.P.; Barrett, K.L.; Campbell P.J.; Forster, R.; Grandy, N.; Huet, M.C.; Lewis, G.; Oomen, P.A.; Schmuck, R. & Vogt, H. (2000): Guidance Document on regulatory testing and risk assessment procedures for plant protection products with non-target arthropods. From the ESCORT 2 workshop, Wageningen, NL.

3.5 REFERENCE LIST

Section identity, physical chemical and analytical methods

Section data on application and efficacy

Section toxicology

Ogbe, Raphael. (2015). A Review of Dietary Phytosterols: Their occurences, metabolism and health benefits. Asian Journal of Plant Science and Research. 5. 10 - 21.

Section residue and consumer risk assessment

Section fate and behavior in environment

Chen, S., Shi, L., Shan, Z., Hu, Q. (2005). Characteristics Of Hydrolysis And Degradation Of Brassinolide In Soils. Rural Eco-Environment, 2005, 21 (1), 55-57.

Section ecotoxicology

None.