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24-Epibrassinolide

Volume 3 – B.9 (PPP) – Sunergist

Rapporteur Member State: Austria

Version History

When	What
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B.9. ECOTOXICOLOGY DATA AND ASSESSMENT OF RISKS FOR NON-TARGET SPECIES

This document contains the ecotoxicological risk assessment for the active substance 24-Epibrassinolide and the product Sunergist, which is a soluble liquid (SL) formulation with active substance content of 0.01% w/w. The representative formulation Sunergist is intended for use as a plant activator/elicitor against fungal diseases in grapes, lettuce, sugarbeet and cucurbits (only glasshouse).

For the inclusion of the active substance 24-Epibrassinolide and the representative formulation Sunergist (0.01 % 24-Epibrassinolide) in Annex I, data and risk assessments to support the application for inclusion regarding ecotoxicology are provided in the following section. Studies, where available, are summarised under the respective data points. In some cases, public literature is used to address data points. In those cases where published literature is used to **address a data point**, an extended summary of the published literature is provided and cited (author, year), the full bibliographical information can be found under point B.9.15.

In the case where published literature is used to **scientifically justify** why a study was not deemed necessary to be conducted or as supporting information, only a superscript is referenced in the text, while full bibliographical information can be found in a respective footnote.

The applicant submitted an extensive introduction to brassinosteroids in support with the dossier of 24-Epibrassinolide. This general information was not evaluated in detail by RMS because it is not deemed necessary for the DAR preparation but is provided in Vol. 3 CA B9 under Appendix I for completeness. Nonetheless a short version was extracted and accepted by RMS and is presented below to give an overview.

B.9.0. INTRODUCTION

Brassinosteroids, including 24-Epibrassinolide are naturally occurring, plant growth promoting molecules, present in higher plants, lower plants, including algae, mosses, the "living fossil" *Equisetum* as well as some fungi.[1,2,3] 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.[4,5]

Brassinosteroids are essential for normal plant growth and development. Those 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.[6] 24-Epibrassinolide elicits and activates the plant's

[1] KCA 8/0001: Takatsuto, S., Abe, H., Gamoah, K. (1990): EVIDENCE FOR BRASSINOSTEROIDS IN STROBILUS OF EQUISETUM ARVENSE L. Report No.: na (092-059) Agricultural and Biological Chemistry, 1990, 54 (4), 1057-1059; Not GLP, published

[2] KCA 8/0011: Bajguz, A., Tretyn, A. (2003): THE CHEMICAL STRUCTURES AND OCCURRENCE OF BRASSINOSTEROIDS IN PLANTS. Report No.: na (092-145). Brassinosteroids. Chapter 1, 2003, 1-44. Not GLP, published.

[3] KCA 8/0012: Bajguz, A. (2011): BRASSINOSTEROIDS – OCCURRENCE AND CHEMICAL STRUCTURES IN PLANTS. In: Hayat, S., Ahmad, A.: BRASSINOSTEROIDS: A CLASS OF PLANT HORMONE. Report No.: na (092-146). Springer Verlag, 2011, Chapter 1, 1-27, DOI 10.1007/978-94-007-0189-2_1; ISBN: 978-94-007-0188-5. Not GLP, published

[4] KCA 8/0002: Zhu, J.-Y., Sae-Seaw, J., Wang, Z.-Y. (2013): BRASSINOSTEROID SIGNALLING. Report No.: na (092-165). Development, 2013, 140(8), 1615-1620; doi: 10.1242/dev.060590. Not GLP, published.

[5] KCA 8/0012: Codreanu, M.; Russinova, E. (2011): REGULATORY MECHANISMS OF BRASSINOSTEROID SIGNALING IN PLANTS. In: Hayat, S., Ahmad, A. (eds.): BRASSINOSTEROIDS: A CLASS OF PLANT HORMONE. Report No.: na (092-146). Springer Verlag, 2011, Chapter 2, 29-56, DOI 10.1007/978-94-007-0189-2_2; ISBN: 978-94-007-0188-5. Not GLP, published

[6] KCA 8/0005: Kutschera, U., Wang, Z.-Y. (2012): BRASSINOSTEROID ACTION IN FLOWERING PLANTS: A DARWINIAN PERSPECTIVE. Report No.: na (092-036). Journal of Experimental Botany, 2012, 63 (10), 3511-3522; doi:10.1093/jxb/ers065. Not GLP, published

self-defence mechanisms mediating the plant's resistance to unfavourable environmental conditions, (e.g. salinity, drought, cold and heat stress) and fungal diseases.[7] Application of brassinosteroids leads to a complex sequence of biochemical reactions such as activation or suppression of key enzymatic reactions, induction of protein synthesis and the production of various chemical defence compounds.[8]

The use pattern for the representative formulation Sunergist (0.01% a.s. w/w) is summarised in Table 9.0-1.

Table 9.0-1 : Intended application pattern (all uses applied via spraying)

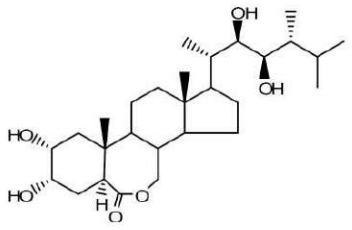
Field use						
Member states	**Crop**	**Timing of application**	**No. of applications**	**Application interval [days]**	**Max. application rate (product) per appl. [L prod./ha]**	**Max. application rate (active substance) per appl. [g a.s./ha]**
All MS	Grapes[1]	15 - 85	3	7	0.5	0.05
All MS	Sugar beet[1]	12 - 39	3	7	0.4	0.04
All MS	Lettuce[2]	10 - 41	2	7	0.4	0.04
Glasshouse use						
Member states	**Crop**	**Timing of application**	**No. of applications**	**Application interval [days]**	**Max. application rate (product) per appl. [L prod./ha]**	**Max. application rate (active substance) per appl. [g as/ha]**
All MS	Cucurbits[2]	12 - 69	3	7	0.5	0.05
All MS	Lettuce[2]	10 - 41	2	7	0.4	0.04

[1] Only professional use

[2] Professional and amateur use

The use of 3 x 0.05 g a.s./ha in grapes was identified to be the critical use for the ecotoxicological risk assessment, covering all other uses if not stated otherwise.

Table 9.0-2 : Substances and metabolites of environmental relevance (structure, synonyms and codes)

Code	**IUPAC name**	**Compound found in**	**Structural formula**
24-Epibrassinolide	(22R,23R,24R)-2α,3α,22,23-tetrahydroxy-24-methyl-β-homo-7-oxa-5-cholestan-6-one	Environment (soil, surface water), plant, rat	
No relevant metabolites. Due to the natural occurrence of 24-Epibrassinolide and its metabolites, risk assessments for metabolites are not considered necessary as they are deemed to be covered by the parent.			

[7] KCA 8/0012: Kang, Y., Guo. S. (2011): ROLE OF BRASSINOSEROIDS ON HORTICULTURAL CROPS. In: Hayat, S., Ahmad, A. (eds.): BRASSINOSTEROIDS: A CLASS OF PLANT HORMONE. Report No.: na (092-146). Springer Verlag, 2011, Chapter 9, 269-288, DOI 10.1007/978-94-007-0189-2_9; ISBN: 978-94-007-0188-5. Not GLP, published

[8] KCA 8/0091: Bajguz, A., Hayat, S. (2009): EFFECTS OF BRASSINOSTEROIDS ON THE PLANT RESPONSES TO ENVIRONMENTAL STRESSES. Report No.: na (092-133). Plant Physiology and Biochemistry, 2009, 47, 1-8; doi:10.1016/j.plaphy.2008.10.002. Not GLP, published

B.9.1. EFFECTS ON BIRDS AND OTHER TERRESTRIAL VERTEBRATES

B.9.1.1. Effects on birds

Toxicity of the active substance

As reported in Vol. 3 CA B9, no toxicity from the active ingredient 24-Epibrassinolide is expected to birds due to its ubiquitous occurrence in plants and the natural exposure of birds to it through their diet.
This is further supported by the public literature Nasonov *et al.* (2016, reference KCA 8.1.1/0001, evaluated in Vol. 3 CA B9 under point B.9.1.1) who studied the immunostimulatory properties of Bravidefen, a brassinosteroid-based drug (24-Epibrassinolide in water-soluble form) in chicken. Bravidefen showed immunostimulatory properties in chicken compared to vaccination only. No effects on behaviour or toxicity were reported at the used dose which equals 0.099163 mg Bravidefen/kg bw (based on the assumption that Bravidefen and 24-Epibrassinolide have the same molecular weight of 396.65 g/mol), underlining that 24-Epibrassinolide can be considered not harmful to birds.

For the active substance an acute and a long-term study in rats was provided by the applicant, for further details please refer to Vol. 3 CA B9 under point B.9.1.2. In light of the low toxicity of the active ingredient and the formulation to mammals, no studies for birds were considered necessary. 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.

Toxicity of the formulated product

No acute effect studies on birds with the product Sunergist were provided by the applicant. The risk assessment will be based on the acute effect product study with rats (LD_{50} > 5000 mg prod./kg bw/d), for further details please refer to the toxicology section in Vol. 3 CP B6.

Table 9.1-1: Toxicity of 24-Epibrassinolide to birds (rat used as surrogate)

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 bridging to birds	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
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

Endocrine disruption

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.

Metabolites of 24-Epibrassinolide

There are no ecotoxicologically relevant metabolites. All metabolites and degradation products of 24-Epibrassinolide are commonly found in plants, soil, surface water and sediment where they usually occur at levels often higher than those that might result from the use of Sunergist. Thus, no risk assessment for metabolites is considered necessary.

B.9.1.2. Effects on terrestrial vertebrates other than birds

Toxicity of the active substance

As reported in Vol. 3 CA B9, no toxicity from the active ingredient 24-Epibrassinolide is expected to mammals due to its ubiquitous occurrence in plants and the natural exposure of mammals to it through feed. However, nonetheless the applicant provided an acute and a long-term effect study on rat with the active substance, for further details please refer to Vol. 3 CA B9 under point B.9.1.2.

Toxicity of the formulated product

An acute effect study on rats with the product Sunergist was provided by the applicant. The risk assessment will be based on this acute effect product study by [redacted] (2017) with LD_{50} > 5000 mg prod./kg bw/d, for further details please refer to the toxicology section in Vol. 3 CP B6.

Table 9.1-2: Toxicity of 24-Epibrassinolide to 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.

Endocrine disruption

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 – B 9 under point B.9.1.5.

Metabolites of 24-Epibrassinolide

There are no ecotoxicologically relevant metabolites. All metabolites and degradation products of 24-Epibrassinolide are commonly found in plants, soil, surface water and sediment where they usually occur at levels often higher than those that might result from the use of Sunergist. Thus, no risk assessment for metabolites is considered necessary.

B.9.2. RISK ASSESSMENT FOR BIRDS AND OTHER TERRESTRIAL VERTEBRATES

Birds and other terrestrial vertebrates may be exposed to 24-Epibrassinolide by eating contaminated vegetation, seeds and fruits, invertebrate prey like arthropods (i.e. insects) or earthworms or vertebrate prey. Another possible route is via drinking water. 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).

Sunergist is intended to be applied as field use on grapes (3 x 0.05 g a.s./ha), sugar beet (3 x 0.04 g a.s./ha), lettuce (2 x 0.04 g a.s./ha) as well as greenhouse use on cucurbits (3 x 0.05 g a.s./ha) and lettuce (2 x 0.04 g a.s./ha).

It is highlighted that 24-Epibrassinolide is omnipresent in nature. Birds and mammals consume brassinosteroids naturally through food intake. Due to the natural exposure to 24-Epibrassinolide and the common occurrence of sterols (e.g. the structurally closely related cholesterol) in animal metabolism it can be concluded that the application of Sunergist is very unlikely to present a significant hazard to birds and mammals. However illustrative risk assessments for birds and mammals are presented below.

B.9.2.1. Risk assessment for birds

For the greenhouse uses no risk assessment was prepared separately since no exposure to birds is expected. It is mentioned that the glasshouse uses are covered by the field uses.

Acute toxicity to birds – Screening step

The acute risk assessment is based on the mammalian acute toxicity endpoint/10 of the active substance (LD_{50} > 5000 mg a.s./kg bw) and of the product (LD_{50} > 0.5 mg a.s./kg bw).

Table 9.2-1: 24-Epibrassinolide acute screening step for birds in grapes (3 x 0.05 g a.s./ha)

Active substance: 24-Epibrassinolide						
Crop	**Indicator species**	**SV_A**	**MAF_{90}**	**DDD_A**	**LD_{50} [mg a.s./kg bw/d]**	**TER**
Vineyard	Small omnivorous bird	95.3	1.6	0.00762	> 500[1]	> 65617
Product: Sunergist (0.01% 24-Epibrassinolide SL)						
Crop	**Indicator species**	**SV_A**	**MAF_{90}**	**DDD_A**	**LD_{50} [mg a.s./kg bw/d]**	**TER**
Vineyard	Small omnivorous bird	95.3	1.6	0.00762	> 0.05[1]	**> 6.56**

Values in **bold** are below the trigger of 10

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

The TER_A value of the active substance is above the trigger of 10 for acute exposure via application in grapes, 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.

Table 9.2-2: 24-Epibrassinolide acute screening step for birds in sugar beet (3 x 0.04 g a.s./ha), also covering the use in lettuce (2 x 0.04 g a.s./ha)

Active substance: 24-Epibrassinolide						
Crop	**Indicator species**	**SV_A**	**MAF_{90}**	**DDD_A**	**LD_{50} [mg a.s./kg bw/d]**	**TER**
Sugar beet	Small omnivorous bird	158.8	1.6	0.01016	> 500[1]	> 49213
Product: Sunergist (0.01% 24-Epibrassinolide SL)						
Crop	**Indicator species**	**SV_A**	**MAF_{90}**	**DDD_A**	**LD_{50} [mg a.s./kg bw/d]**	**TER**
Sugar beet	Small omnivorous bird	158.8	1.6	0.01016	> 0.05[1]	> 4.92

Values in **bold** are below the trigger of 10

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

The TER_A value of the active substance is above the trigger of 10 for acute exposure via application in sugar beet, 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.

Acute toxicity to birds – Tier 1

Table 9.2-3: Sunergist (0.01% 24-Epibrassinolide SL) acute Tier 1 risk assessment for birds in grapes (3 x 0.05 g a.s./ha) at BBCH 15 - 85

Crop	Indicator species	SV_A	MAF_{90}	DDD_A	LD_{50} [mg a.s./kg bw/d]	TER
BBCH 10 - 19	Small insectivorous bird "Redstart"	27.4	1.6	0.00219	> 0.05[1]	> 23
BBCH ≥ 20	Small insectivorous bird "Redstart"	25.7		0.00206		> 24
BBCH 10 - 19	Small granivorous bird "Finch"	14.8		0.00118		> 42
BBCH 20 - 39	Small granivorous bird "Finch"	12.4		0.00099		> 50
BBCH ≥ 40	Small granivorous bird "Finch"	7.4		0.00059		> 84
Ripening	Frugivorous bird "Thrush"	28.9		0.00231		> 22
BBCH 10 - 19	Small omnivorous bird "lark"	14.4		0.00115		> 43
BBCH 20 - 39	Small omnivorous bird "lark"	12.0		0.00096		> 52
BBCH ≥ 40	Small omnivorous bird "lark"	7.2		0.00058		> 87

Values in **bold** are below the trigger of 10

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

The Tier 1 acute TER values are above the trigger of 10 for acute exposure via application in grapes, therefore an acceptable risk to birds is demonstrated for the proposed use of the product Sunergist. 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 a in-depth bridging argumentation. Further 0% mortality occured in the acute rat toxicity study, thus this endpoint can be considered conservative.

Table 9.2-4: Sunergist (0.01% 24 Epibrassinolide-SL) acute Tier 1 risk assessment birds in <u>sugar beet</u> (3 x 0.04 g a.s./ha) at BBCH 10 – 41, also covering the use in lettuce (2 x 0.04 g a.s./ha)

Crop	Indicator species	SV_A	MAF_{90}	DDD_A	LD_{50} [mg a.s./kg bw/d]	TER
Early (BBCH 10 – 19)	Small omnivorous bird "lark"	24.0	1.6	0.00154	> 0.05[1]	> 33
Late (BBCH 30 – 49)	Small granivorous bird "Finch"	24.7		0.00158		> 32
BBCH 10 - 19	Small insectivorous bird "wagtail"	10.9		0.00070		> 72
BBCH 20 - 49	Small insectivorous bird "wagtail"	7.7		0.00049		> 101
BBCH 10 - 19	Small insectivorous bird "wagtail"	10.9		0.00070		> 72
BBCH 20 - 49	Small insectivorous bird "wagtail"	25.2		0.00161		> 31

Values in **bold** are below the trigger of 10

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

The Tier 1 acute TER values are above the trigger of 10 for acute exposure via application in sugar beet, therefore an acceptable risk to birds is demonstrated for the proposed use of the product Sunergist. 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 occurred in the acute rat toxicity study, thus this endpoint can be considered conservative.

<u>Long-term toxicity to birds – Screening step</u>

The acute risk assessment is based on the mammalian toxicity endpoint/10 (NOEL = 1000 mg a.s./kg bw).

Table 9.2-5: 24-Epibrassinolide long-term screening step for birds in <u>grapes</u> (3 x 0.05 g a.s./ha)

Crop	Indicator species	SV_{LT}	MAF_m	TWA	DDD_{LT}	NOEL [mg a.s./kg bw/d]	TER
Vineyard	Small omnivorous bird	38.9	2.0	1[2]	0.00389	100[1]	25707

Values in **bold** are below the trigger of 10

[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 long-term endpoint was derived in a OECD TG 414 study in rats with an exposure period of 14 days, therefore as worst case assumption the toxic effect is considered to be caused by short-term exposure and the TWA is set to 1

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.

Table 9.2-6: 24-Epibrassinolide long-term screening step for birds in <u>sugar beet</u> (3 x 0.04 g a.s./ha), also covering the use in lettuce (2 x 0.04 g a.s./ha)

Crop	Indicator species	SV_{LT}	MAF_m	TWA	DDD_{LT}	NOEL [mg a.s./kg bw/d]	TER
Sugar beet	Small omnivorous bird	64.8	2.0	1[2]	0.00518	100[1]	19305

Values in **bold** are below the trigger of 10

[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 long-term endpoint was derived in a OECD TG 414 study in rats with an exposure period of 14 days, therefore as worst case assumption the toxic effect is considered to be caused by short-term exposure and the TWA iss et to 1

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

Risk from bioaccumulation and food chain behaviour

- Secondary poisoning

According to the Guidance Document (EFSA, 2009), substances with a log Pow ≥ 3 have potential for bioaccumulation and should be assessed for the risk of biomagnification in terrestrial food chains.
As 24-Epibrassinolide has a log Pow of 2.0, no bioaccumulations is expected (please refer to Vol. 3 CA Part B 2). Therefore, no risk assessment on food chain behaviour is considered necessary.

- Food chain from earthworm to earthworm-eating birds

Not relevant since log Pow of 24-Epibrassinolide is below the trigger value of 3.

- Food chain from fish to fish-eating birds

Not relevant since log Pow of 24-Epibrassinolide is below the trigger value of 3.

- Biomagnification in terrestrial food chains

Not relevant since log Pow of 24-Epibrassinolide is below the trigger value of 3.

Risk from consumption of contaminated water

The risk to birds from exposure via drinking water was conducted according to the EFSA Guidance Document on Birds and Mammals (2009). Due to the nature of 24-Epibrassinolide, free 24-Epibrassinolide is absorbed by plants almost immediately after application. Further, only very small amounts of the active substance (maximum of 0.05 g a.s/ha in grape) are applied. This is why no significant amounts of the a.s. are expected to be held in axils of leaves of treated plants or in puddles of spray liquid on the soil. Nonetheless a risk assessment is presented below.

Leaf scenario (for lettuce):

For the leaf scenario calculation the concentration of the active substance mixed with water in the use on lettuce (0.04 g a.s./200 L water) is used, i.e. C_{spray} = 0.2 mg a.s./L water.

$$PEC_{pool} = \frac{C_{spray}}{5}$$

$$TER_{dw} = \frac{Endpoint}{PEC_{pool} \times DWR}$$

Table 9.2-7: Leaf water scenario risk assessment of 24-Epibrassinolide uptake for birds in lettuce (2 x 0.04 g a.s./ha)

C_{spray} [mg a.s./L water]	PEC_{Pool} [mg a.s./L]	DWR [L/kg bw/d]	PEC_{Pool} x DWR	Acute endpoint [mg a.s./kg bw/d]	TER_{dw}	Trigger
0.2	0.04	0.46	0.0184	> 500[1]	> 27174	10

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

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.

Puddle scenario:

According to the EFSA Guidance Document for birds and mammals (2009), no specific calculations of exposure via puddle scenario are necessary, when the ratio of effective application rate (in g/ha) to relevant endpoint (in mg/kg bw/d) does not exceed 50 in the case of less sorptive substances (Koc < 500 L/kg). For PECsw calculations, a worst case Koc of 0 for 24-Epibrassinolide was assumed (see Vol. 3 CP B8), which is also considered valid for this exposure scenario. The application rate of the proposed use in grape (0.05 g a.s./ha) covers all other uses.

$$\frac{Application\ rate\ [g\ a.s./ha]}{Endpoint\ [mg\ a.s./kg\ bw/d]} < 50$$

Table 9.2-8: Puddle water scenario risk assessment of 24-Epibrassinolide uptake for birds in grapes (3 x 0.05 g a.s./ha), covering all other uses

	Application rate [g a.s./ha]	Endpoint [mg a.s./kg bw/d]	Ratio [Application rate/Endpoint]	Trigger
Acute	0.05	> 500[1]	< 0.0001	50[2]
Long-term	0.05	100[1]	0.0005	50[2]

[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 trigger value of 50 is applicable for substances with Koc < 500 L/kg, for 24-Epibrassinolide a Koc of 0 in water was assumed in Vol. 3 CP B8

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.

The evaluation of leaf scenario and puddle scenario confirms that the risk for birds from drinking water that may contain residues from the use of the product is acceptable. It is noted that the application rate of the proposed use in grape (3 x 0.05 g a.s./ha) covers all other uses.

Consideration of metabolites

- Screening level risk assessment for metabolites

There are no ecotoxicologically relevant metabolites. All metabolites and degradation products of 24-Epibrassinolide are commonly found in plants, soil, surface water and sediment where they usually occur at levels often higher than those that might result from the use of Sunergist. Thus, no risk assessment for metabolites is considered necessary.

- Bioaccumulation risk assessment for metabolites

There are no ecotoxicologically relevant metabolites. All metabolites and degradation products of 24-Epibrassinolide are commonly found in plants, soil, surface water and sediment where they usually occur at levels often higher than those that might result from the use of Sunergist. Thus, no risk assessment for metabolites is considered necessary.

Identification of endocrine disrupting properties

There is at present no indication that 24-Epibrassinolide act as an endocrine disruptor.
Please refer for more details to Vol. 3 CA B9 under point B.9.1.5.

B.9.2.2. Risk assessment for mammals

Acute toxicity to mammals – Screening step

The acute risk assessment is based on the mammalian acute toxicity endpoint of the active substance (LD_{50} > 5000 mg a.s./kg bw) and of the product (LD_{50} > 0.5 mg a.s./kg bw).

Table 9.2-9: 24-Epibrassinolide acute screening step for mammals in grapes (3 x 0.05 g a.s./ha), also covering all other uses

Active substance: 24-Epibrassinolide						
Crop	**Indicator species**	**SV_A**	**MAF_{90}**	**DDD_A**	**LD_{50} [mg a.s./kg bw/d]**	**TER**
Vineyard	Small herbivorous mammal	136.4	1.6	0.0109	> 5000	> 458716
Product: Sunergist (0.01% 24-Epibrassinolide SL)						
Crop	**Indicator species**	**SV_A**	**MAF_{90}**	**DDD_A**	**LD_{50} [mg a.s./kg bw/d]**	**TER**
Vineyard	Small herbivorous mammal	136.4	1.6	0. 0109	> 0.5	> 46

Values in **bold** are below the trigger of 10

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

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.

Acute toxicity to mammals – Tier 1

Not required.

Long-term toxicity to mammals

The acute risk assessment is based on the mammalian toxicity endpoint (NOEL = 1000 mg a.s./kg bw).

Table 9.2-10: 24-Epibrassinolide long-term screening step for mammals in grapes (3 x 0.05 g a.s./ha), also covering all other uses

Crop	**Indicator species**	**SV_{LT}**	**MAF_m**	**TWA**	**DDD_{LT}**	**NOEL [mg a.s./kg bw/d]**	**TER**
Vineyard	Small herbivorous mammal	72.3	2.0	1[1]	0.00723	1000	138313

Values in **bold** are below the trigger of 10

[1] The long-term endpoint was derived in a OECD TG 414 study in rats with an exposure period of 14 days, therefore as worst case assumption the toxic effect is considered to be caused by short-term exposure and the TWA is set to 1

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.

Risk from bioaccumulation and food chain behaviour

- Secondary poisoning

According to the Guidance Document (EFSA, 2009), substances with a log Pow ≥ 3 have potential for bioaccumulation and should be assessed for the risk of biomagnification in terrestrial food chains.
As 24-Epibrassinolide has a log Pow of 2.0, no bioaccumulations is expected (please refer to Vol. 3 CA Part B 2). Therefore, no risk assessment on food chain behaviour is considered necessary.

- Food chain from earthworm to earthworm-eating birds

Not relevant since log Pow of 24-Epibrassinolide is below the trigger value of 3.

- Food chain from fish to fish-eating birds

Not relevant since log Pow of 24-Epibrassinolide is below the trigger value of 3.

- Biomagnification in terrestrial food chains

Not relevant since log Pow of 24-Epibrassinolide is below the trigger value of 3.

Risk from consumption of contaminated water

The risk to mammals from exposure via drinking water was conducted according to the EFSA Guidance Document on Birds and Mammals (2009). Due to the nature of 24-Epibrassinolide, free 24-Epibrassinolide is absorbed by plants almost immediately after application. Further, only very small amounts of the active substance (maximum of 0.05 g a.s/ha in grape) are applied. This is why no significant amounts of the a.s. are expected in puddles of spray liquid on the soil. Nonetheless a risk assessment is presented below.

Puddle scenario:

According to the EFSA Guidance Document for birds and mammals (2009), no specific calculations of exposure via puddle scenario are necessary, when the ratio of effective application rate (in g/ha) to relevant endpoint (in mg/kg bw/d) does not exceed 50 in the case of less sorptive substances (Koc < 500 L/kg). For PECsw calculations, a worst case Koc of 0 for 24-Epibrassinolide was assumed (see Vol. 3 CP B8), which is also considered valid for this exposure scenario. The application rate of the proposed use in grape (0.05 g a.s./ha) covers all other uses.

$$\frac{Application\ rate\ [g\ a.s./ha]}{Endpoint\ [mg\ a.s./kg\ bw/d]} < 50$$

Table 9.2-11: Puddle water scenario risk assessment of 24-Epibrassinolide uptake for mammals in grapes (3 x 0.05 g a.s./ha), covering all other uses

	Application rate [g a.s./ha]	Endpoint [mg a.s./kg bw/d]	Ratio [Application rate/Endpoint]	Trigger
Acute	0.05	> 5000	< 0.00001	50[1]
Long-term	0.05	1000	0.00005	50[1]

[1] The trigger value of 50 is applicable for substances with Koc < 500 L/kg, for 24-Epibrassinolide a Koc of 0 in water was assumed in Vol. 3 CP B8

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.

The evaluation of the puddle scenario confirms that the risk for mammals from drinking water that may contain residues from the use of the product is acceptable. It is noted that the application rate of the proposed use in grape (3 x 0.05 g a.s./ha) covers all other uses.

Consideration of metabolites

- Screening level risk assessment for metabolites

There are no ecotoxicologically relevant metabolites. All metabolites and degradation products of 24-Epibrassinolide are commonly found in plants, soil, surface water and sediment where they usually occur at levels often higher than those that might result from the use of Sunergist. Thus, no risk assessment for metabolites is considered necessary.

- Bioaccumulation risk assessment for metabolites

There are no ecotoxicologically relevant metabolites. All metabolites and degradation products of 24-Epibrassinolide are commonly found in plants, soil, surface water and sediment where they usually occur at levels often higher than those that might result from the use of Sunergist. Thus, no risk assessment for metabolites is considered necessary.

Identification of endocrine disrupting properties

There is at present no indication that 24-Epibrassinolide act as an endocrine disruptor.
Please refer for more details to Vol. 3 CA B9 under point B.9.1.5.

B.9.3. EFFECTS ON AQUATIC ORGANISMS

Toxicity of the active substance

No studies with the EU representative formulation Sunergist have been conducted with fish, aquatic invertebrates, algae and aquatic plants. However, active substance data for fish and aquatic invertebrates is available for 24-Epibrassinolide.

Aquatic organisms may be exposed to 24-Epibrassinolide by emissions from treated fields. The provided studies and data permit a risk assessment for 24-Epibrassinolide under practical conditions. Relevant study summaries as well as the applicants' waivers for the active substance effect studies on sediment-dwelling organisms, algae and macrophyts can be found in Vol. 3 CA B9 under point B.9.2. The effect studies of the active substance on algae and macrophyts were waived, however both aquatic plants are considered to be potentially susceptible organisms (due to possible effects on growth under static exposure laboratory conditions). Since such potential effects are considered unlikely but can't be fully excluded it is proposed to classify 24-Epibrassinolide according to Regulation (EU) 286/2011 within the "safety net" as hazard class "aquatic chronic 4 (H413)" since no studies are presented and the active substance is considered as not readily biodegradable (please refer to the RMS fate comment in Vol. 3 CA B8 under point 7.2.2.1).

Table 9.3-1: Toxicity data of 24-Epibrassinolide for aquatic species

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)
No long-term data for fish submitted. Please refer to Vol. 3 CA B9 B.9.2.2 for the waiver.					
Aquatic invertebrates					
Daphnia magna	24-Epibrassinolide	Acute, 48 hr (static)	Mortality, EC_{50}	> 2.86 (mm)	Matlock, D. & Moore, S. (2017)
No long-term data for aquatic invertebrates submitted. Please refer to Vol. 3 CA B9 B.9.2.5 for the waiver.					
Sediment-dwelling organisms					
No data submitted. Please refer to Vol. 3 CA B9 B.9.2.5.3 for the waiver.					
Algae					
No data submitted. Please refer to Vol. 3 CA B9 B.9.2.6 for the waiver.					
Higher plant					
No data submitted. Please refer to Vol. 3 CA B9 B.9.2.7 for the waiver.					
Further testing on aquatic organisms					
Not required.					
Potential endocrine disrupting properties					
No ED potential indicated. Please refer to Vol. 3 B9 B.9.2.3 for further details.					

(nom)…nominal concentration; (mm)…mean measured concentration

Metabolites of 24-Epibrassinolide

There are no ecotoxicologically relevant metabolites. All metabolites and degradation products of 24-Epibrassinolide are commonly found in plants, soil, surface water and sediment where they usually occur at levels often higher than those that might result from the use of Sunergist. Thus, no risk assessment for metabolites is considered necessary.

B.9.3.1. Acute toxicity to fish, aquatic invertebrates, or effects on aquatic algae and macrophytes

The applicant submitted the following statement:

"No studies with the formulation were performed, as it is possible to extrapolate effects from the studies undertaken with the active substance. This is supported by the fact that toxicity was neither found in an acute active substance study nor in an acute product study with mammals, indicating that the product is not more toxic

than the active substance. Further, no co-formulants of concern to aquatic organisms are part of the formulated product in significant amounts (see [Vol. 4 Part C]*). Therefore, no toxicity from these co-formulants is expected, also due to the fact that only up to 1.5 L product/ha is used in total and the environmental concentration of the co-formulants will be very low. Further, all co-formulants are readily biodegradable. For further information, please refer to* [Vol. 4 Part C].
This is why the acute toxicity study to fish and to Daphnia magna with 24-Epibrassinolide is considered sufficient to address this point. Please refer to [Vol. 3 CA B9 B.9.2.1 and Vol. 3 CA B9 B.9.2.4.1]*, respectively for more details on the active substance studies."*

Comment RMS:
No studies with the product Sunergist have been submitted by the applicant. The formulation Sunergist contains the active ingredient (0.01% w/w), mostly non-toxic co-formulants, but also a small portion of co-formulants with aquatic hazard classification according CLP (see Vol. 4 Part C). Therefore, a risk assessment for the active substance will be presented for the available endpoints (fish acute and aquatic invertebrates acute).

Acute toxicity of the product to fish
No studies with the product Sunergist have been conducted by the applicant. The formulation Sunergist contains the active ingredient (0.01% w/w), mostly non-toxic co-formulants, but also a small portion of co-formulants with aquatic hazard classification according CLP (see Vol. 4 Part C). Nonetheless, it is considered acceptable to base the risk assessment on the active substance with the Zebrafish (*Danio rerio*) endpoint of LC_{50} (96 hr) > 5.0 mg a.s./L (nom).

Acute toxicity of the product to aquatic invertebrates
No studies with the product Sunergist have been conducted by the applicant. The formulation Sunergist contains the active ingredient (0.01% w/w), mostly non-toxic co-formulants, but also a small portion of co-formulants with aquatic hazard classification according CLP (see Vol. 4 Part C). Nonetheless, it is considered acceptable to base the risk assessment on the active substance with the *Daphnia magna* endpoint of EC_{50} (48 hr) > 2.86 mg a.s./L (mm).

Effects of the product on aquatic algae and macrophyts
No studies with the product Sunergist have been conducted by the applicant. The formulation Sunergist contains the active ingredient (0.01% w/w), mostly non-toxic co-formulants, but also a small portion of co-formulants with aquatic hazard classification according CLP (see Vol. 4 Part C). The effect studies of the active substance on algae and macrophyts were waived, however aquatic plants are considered to be potentially susceptible organisms (due to possible effects on growth under static exposure laboratory conditions).

B.9.3.2. Additional long-term and chronic toxicity studies on fish, aquatic invertebrates and sediment dwelling organisms

Chronic toxicity of the product to fish
No studies with the product Sunergist have been conducted. A waiver of long-term studies with the active substance 24-Epibrassinolide in fish was requested by the applicant and considered as reasonable by RMS. Please refer to Vol. 3 CA B9 point B.9.2.2. A qualitative risk consideration is presented below under point B.9.4.2.

Chronic toxicity of the product to aquatic invertebrates
No studies with the product Sunergist have been conducted. A waiver of long-term studies with the active substance 24-Epibrassinolide in fish was requested by the applicant and considered as reasonable by RMS. Please refer to Vol. 3 CA B9 point B.9.2.5. A qualitative risk consideration is presented below under point B.9.4.2.

Chronic toxicity of the product to sediment-dwelling organisms

No studies with the product Sunergist have been conducted. A waiver of long-term studies with the active substance 24-Epibrassinolide in fish was requested by the applicant and considered as reasonable by RMS. Please refer to Vol. 3 CA B9 point B.9.2.5.3. A qualitative risk consideration is presented below under point B.9.4.2.

B.9.3.3. Further testing on aquatic organisms

Not considered necessary.

B.9.4. RISK ASSESSMENT FOR AQUATIC ORGANISMS

The risk assessment was carried out according to EFSA Journal 2013;11(7):3290. Regulatory acceptable concentrations (RACs) were derived for each available aquatic endpoint by dividing the endpoint by an assessment factor. The RAC was then compared to the PEC_{sw} through the ratio PEC/RAC. In case this ratio is below 1, an acceptable risk is indicated, as the predicted environmental concentration is below the regulatory acceptable concentration. Results of the risk assessment are presented in Table 9.4-3.

Endpoints used for the risk assessment

Table 9.4-1: Endpoints: Toxicity to fish and aquatic invertebrates, effects on algae

Group	Test substance	Time scale	Endpoint	Endpoint [mg a.s./L]	Reference
Fish					
Zebrafish (*Danio rerio*)	24-Epibrassinolide	Acute, 96 hr, static	Mortality, LC_{50}	**> 5.0 (nom)**	[redacted] (2017)
Aquatic invertebrates					
Daphnia magna	24-Epibrassinolide	Acute, 48 hr, static	Mortality, EC_{50}	**> 2.86 (mm)**	Matlock, D. & Moore, S. (2017)

Values in **bold** are used for the risk assessment

Metabolites of 24-Epibrassinolide

There are no ecotoxicologically relevant metabolites. All metabolites and degradation products of 24-Epibrassinolide are commonly found in plants, soil, surface water and sediment where they usually occur at levels often higher than those that might result from the use of Sunergist. Thus, no risk assessment for metabolites is considered necessary.

Endpoints of the product Sunergist

No studies with the product Sunergist have been submitted by the applicant. The formulation Sunergist contains the active ingredient (0.01% w/w), mostly non-toxic co-formulants, but also a small portion of co-formulants with aquatic hazard classification according CLP (see Vol. 4 Part C). Therefore, a risk assessment for the active substance will be presented for the available endpoints (fish acute and aquatic invertebrates acute).

Predicted environmental surface water concentrations (PEC_{SW} and $PEC_{Sediment}$)

The initial PEC_{SW} and PEC_{Sed} of 24-Epibrassinolide in surface water and sediment after use of the plant protection product Sunergist have been assessed according to the EU recommendations for estimating drift entries in surface water (please refer to Vol. 3 CP B8 for further details).

Table 9.4-2: Initial PECsw and PECsed values for 24-Epibrassinolide following the intended uses of Sunergist

Crop	FOCUS Scenario	Application timing	Max. PECsw[1] [µg a.s./L]	Max. PECsed[2] [µg a.s./kg]
Grapes (3 x 0.05 g a.s./ha)	Step 1	late	0.0540	0.3488
	Step 2 (Southern Europe)	March-May	0.0190	0.1337
Sugar beet (3 x 0.04 g a.s./ha)	Step 1	-	0.0411	0.2791
	Step 2 (Southern Europe)	March-May	0.0150	0.1056
Lettuce (2 x 0.04 g a.s./ha)	Step 1	-	0.0274	0.1860
	Step 2 (Northern Europe)	Oct-Febr	0.0129	0.0907
Cucurbits (3 x 0.05 g a.s./ha)	Step 1	-	0.514	0.3488
	Step 2 (Northern Europe)	Oct-Febr	**0.0232**	**0.1632**

[1] For surface water PEC calculation a worst case Koc of 0 was used
[2] For sediment PEC calculation a worst case Koc of 10000 was used
The worst case FOCUS Step 2 PECs used in the risk assessment are presented in **bold**

Acute and long-term risk assessment

In the following chapters, the risk of exposure of aquatic organisms to 24-Epibrassinolide/Sunergist will be quantified by calculating the regulatory acceptable concentration as follows:

$$RAC_{Acute} = \frac{EC_{50}\ or\ LC_{50}}{100}$$

$$RAC_{Long-term} = \frac{NOEC}{10}$$

An acceptable risk is indicated if:

$$\frac{PEC_{sw}}{RAC_{Acute/Long-term}} < 1$$

where: RAC = regulatory acceptable concentration, EC_{50}/LC_{50} = 50 % effective/lethal concentration, NOEC = no observed effect concentration, PEC = predicted environmental concentration, SW = surface water.

B.9.4.1. Acute risk

PEC/RAC$_{Acute}$ ratios of 24-Epibrassinolide

Table 9.4-3: Acute risk to aquatic organisms: acceptability of risk (PEC/RAC < 1) 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 $_{gl-max}$ (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.

B.9.4.2. Chronic risk

No endpoints for a quantitative chronic risk assessment with 24-Epibrassinolide are available, please refer to point B.9.3.2 above for further details. Therefore a qualitative risk consideration for the chronic exposure is presented.

Qualitative chronic risk consideration for fish and aquatic invertebrates

No studies were submitted by the notifier to address the long-term effects on fish and aquatic invertebrates. No explicit data was presented by the applicant regarding natural background concentrations of 24-Epibrassinolide in water. In a public literature study by Hassett et al. (1977, reference KCA 8.2.5.4/0001, evaluated in Vol.3 CA B9 point 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. Although brassinosteroids are not explicitly mentioned, the referenced sterols are considered to have a close structural relation or represent even precursors of brassinosteroids and are therefore seen suitable to serve as a proxy to estimate the order of magnitude of 24-Epibrassinolide concentration in water. A worst case suface 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 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 chronic fish and aquatic invertebrates studies is considered acceptable 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 (please refer to Vol. 3 CA Part B 2) 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.

Qualitative chronic risk considerations for sediment dwelling organisms

No studies were submitted by the notifier to address the effects on sediment dwelling organisms. No explicit data was presented by the applicant regarding natural background concentrations of 24-Epibrassinolide in sediment. In the publication by Hassett et al. (1977) sterols were found in two North American lakes, with lake sediment sterol concentrations in the range of 3 mg/kg sediment dw. Although brassinosteroids are not explicitly mentioned, the referenced sterols are considered to have a close structural relation or represent even precursors of brassinosteroids and are therefore seen suitable to serve as a proxy to estimate the order of magnitude of 24-Epibrassinolide concentration in sediment. A worst case sediment PEC of 0.1632 µg a.s./kg sediment dw was calculated in the fate section in Vol. 3 CP B 8. This supports the argument 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 studies on sediment dwelling organisms is considered acceptable 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 *Daphnia magna* and 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 negative long-term effects to sediment dwelling organisms posed by 24-Epibrassinolide are therefore considered unlikely and an acceptable risk is concluded.

B.9.4.3. Bioconcentration and secondary poisoning

Due to the moderate lipophilic properties of 24-Epibrassinolide with a log Pow = 2.0 (please refer to Vol. 3 CA B2), high bioconcentrations in fish are not to be expected and testing is not considered necessary according to Commission Regulation (EU) 283/2013. 24-Epibrassinolide occurs ubiquitously in plants and is used in fish farms in Russia to improve water quality (Zhabinskii *et al.* (2015), reference KCA 8.2.1/0002, evaluated under Vol. 3 CA B9 B.9.2.1). Brassinosteroids have been found in aquatic organisms such as algae (e.g. *Chlorella vulgaris*) and fish are naturally exposed to these organisms and substances. Therefore the risk of bioconcentration and secondary poisoning is considered negligible.

B.9.5. EFFECTS ON ARTHROPODS

B.9.5.1. Effects on bees

Toxicity of the active substance to bees

Table 9.5-1: Toxicity endpoints of 24-Epibrassinolide in bees

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 of the active substance with adult bees was submitted by the applicant, justification in Vol. 3 CA B9 under point B.9.3.1.2.					
Bee brood development					
No bee brood development study of the active substance was submitted by the applicant, please refer to Vol. 3 CA B9 under point B.9.3.1.2.					
Sub-lethal effects					
No sub-lethal effects study on bees (behavioural and reproductive) of the active substance was submitted by the applicant, please refer to Vol. 3 CA B9 under point B.9.3.1.2.					

B.9.5.1.1. Acute toxicity to bees

Acute oral toxicity of the product to bees

The applicant submitted the following statement:
"No study with the product was carried out. However, data with the active substance is available and considered sufficient, as studies with the formulation on non-target arthropods showed an acceptable [acute] *risk for this organism group. Further, co-formulants in the product are only present in small amounts (see* [Vol. 4 Part C]*) and no toxicity to bees was reported for them. Please refer to CA 8.3.1.1.1 for details on the acute oral toxicity study conducted with the active substance."*

This argument is accepted by RMS and it is concluded that waiving the acute oral toxicity studies with Sunergist on honeybees is considered reasonable, thus active substance data was used for the risk assessment.

Acute contact toxicity of the product to bees

The applicant submitted the following statement:
"No study with the product was carried out. However, data with the active substance is available and considered sufficient, as studies with the formulation on non-target arthropods showed an acceptable [acute] *risk for this organism group. Further, co-formulants in the product are only present in small amounts (see* [Vol. 4 Part C]*) and no toxicity to bees was reported for them. Please refer to CA 8.3.1.1.2 for details on the acute contact toxicity study conducted with the active substance."*

This argument is accepted by RMS and it is concluded that waiving the acute contact toxicity studies with Sunergist on honeybees is considered reasonable, thus active substance data was used for the risk assessment.

B.9.5.1.2. Chronic toxicity to adult bees

Chronic toxicity of the product to adult bees

No formulation studies were submitted by the notifier to address chronic toxicity to adult bees.

The applicant submitted the following statement:
"Due to the ubiquitous occurrence of 24-Epibrassinolide in plants, nectar and pollen, the natural exposure of bees to the substance, as well as the low acute oral and acute contact toxicity for bees of the active substance and the fact that co-formulants in the product are only present in small amounts (see [Vol. 4 Part C]*) without reported toxicity to bees, testing of chronic toxicity to bees is not considered necessary. Further, studies with the formulation on non-target arthropods showed an acceptable risk for this organism group."*

This argument is only partly accepted by RMS. There is evidence, that 24-Epibrassinolide is naturally found in various plants, pollen and honey. According to Ikekawa *et al.* (1988)[9], the highest concentration of 24-Epibrassinolide in bee pollen of *Vicia faba* was 5 µg/kg fresh weight and Khripach *et al.* (2013)[10] reported 7.4 ng 24-Epibrassinolide/g in honey.
The public literature by Chuda-Mickiewicz *et al.* (2009, reference KCA 8.3.1/0001, evaluated in Vol. 3 CA B9 B.9.3.1) 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

[9] KCA 8.3.1/0002: Ikekawa, N., Nishiyama, F., Fujimoto, Y. (1988): IDENTIFICATION OF 24-EPIBRASSINOLIDE IN BEE POLLEN OF THE BROAD BEAN, VICIA FABA L., Report No.: na (092-027), Chemical and Pharmaceutical Bulletin, 1988, 36 (1), 405-407, Not GLP, published

[10] KCA 8.3.1/0003: Khripach, V.A., Litvinovskaya, R.P., Kurtikova, A.L., Drach, S.V., Pryadko, A.G., Mirantsova, T.V., Baranovskiy, A.V. (2013): ENZYME IMMUNOASSAY OF THE CONTENT OF ENDOGENOUS BRASSINOSTEROIDS IN PHYTOGENIC FOOD PRODUCTS, Report No.: na (092-030), National Academy of Sciences of Belarus, 2013, 57 (2), 63-69, Not GLP, published

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.
However, no data was submitted by the applicant 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.

B.9.5.1.3. Effects on honeybee development and other honeybee life stages

Please refer to B.9.5.1.2.

B.9.5.1.4. Cage and tunnel tests

Not considered necessary, please refer to B.9.5.1.2.

B.9.5.1.5. Field tests

Not considered necessary, please refer to B.9.5.1.2.

B.9.5.2. Effects on non-target arthropods other than bees

Toxicity of the active substance to NTAs

Table 9.5-2: Toxicity endpoints of 24-Epibrassinolide in non-target arthropods other than bees

Species	Test substance	Time scale/type of endpoint	End point	Toxicity	Reference
No studies were submitted with the active substance, please refer to Vol. 3 B9 B.9.3.2.					

Toxicity of the product to NTAs
Due to the ubiquitous occurrence of 24-Epibrassinolide in plants and plant parts, non-target arthropods are naturally exposed to the substance through feeding on plant sap, nectar or other plant-sucking insects.
Nevertheless, laboratory studies with the formulation Sunergist (24-Epibrassinolide 0.01% SL) were carried out to assess the toxicity of the formulation to *Aphidius rhopalosiphi* and *Typhlodromus pyri.*

B.9.5.2.1. Effect study on *Aphidius rhopalosiphi*

Data point addressed:	CP B.9.5.2
Reference:	Study was not submitted via CADDY
Author(s) (year):	Moll, M.(2017)
Title:	Sunergist (Epibrassinolide 0.01% Soluble Liquid): Effects on the Parasitoid *Aphidius rhopalosiphi* in the Laboratory - Dose Response Test -
Laboratory report / project	120141001

Number (Doc No.):	
Testing facility:	Ibacon GmbH, Rossdorf, Germany
Published:	No
Test guideline used:	Mead Briggs et al 2000 and Mead-Brigges *et al.* 2010
Deviations:	None
GLP:	Yes
Acceptability:	**Yes**

Executive Summary

The effect of a SL formulation containing 0.01 % Epibrassinolide applied at 5 dose rates (438 - 7000 mL product/ha, corresponding to 0.043 g a.s/L – 0.69 g a.s./ha, on the parasitoid *Aphidius rhopalosiphi* was measured in the laboratory via contact on treated glass surfaces compared to a water treated control and to a reference item. An assessment on mortality seen over 48 h of exposure and for sublethal effects (parasitation activity) compared to a water treated control was performed.
Under worst case laboratory conditions the LR50 of Sunergist (Epibrassinolide 0.01% Soluble Liquid) is estimated to be greater than 7000 mL product/ha in 200 L water/ha (corresponding to > 0.69 g a.s./ha). Reproduction of *Aphidius rhopalosiphi* was assessed in the control and at all test item dose rates. Reproduction was not affected up to and including 7000 mL product/ha, and the ER50 is therefore estimated to be greater than 7000 mL product/ha in 200 L water/ha (corresponding to > 0.69 g a.s./ha).

I. MATERIALS AND METHODS

A. MATERIALS

1. Test Material:	Sunergist (Epibrassinolide 0.01% Soluble Liquid)
Active ingredient:	24-Epibrassinolide
Description:	colorless
Lot/Batch #:	002-20150506
Content of a.s.:	0.01% (w/w)
2. Vehicle and control:	
Control:	Deionised water
Reference Item	Perfekthion (BAS 152 11 I) (420.3 g/L dimethoate)
3. Test animals:	
Species:	*Aphidius rhopalosiphi* (DeStefani-Perez)
Taxonomic group:	Parasitoids (Hymenoptera: Braconidae)
Life Stage:	adults
Age:	Not more than 48 hours
Source:	Katz Biotech AG, An der Birkenpfuhlheide 10, D-15837 Baruth
Acclimation period:	Approximately 1 - 2 days under test conditions in hatching chambers
Environmental conditions:	
Temperature:	18 – 22 °C
Humidity:	73 - 81 % (acclimatisation, exposure period)
	84 - 85 % (post-exposure period; within the test units)
Photoperiod:	16 hours light – 8 hours dark
Light intensity:	910 - 1920 lux (acclimatisation, exposure, parasitisation period)
	8820 - 15380 lux (post-parasitisation period)

In life dates: 10.01.2017 – 07.02.2017

B. STUDY DESIGN AND METHODS

The appropriate amounts of the test item were weighted and dissolved in deionised water to achieve the needed concentrations. The dilutions were sprayed onto glass plates and dried for 25 to 55 minutes, before the introduction of the test animals. During the exposure period, each treatment group consisted of 4 replicates with 10 individuals (7 female, 3 male)

per unit. During the post-exposure period, 20 replicates per treatment group with 1 female per test unit were used. In the post-exposure period, *Aphidius rhopalosiphi* were held in untreated pots with barley seedlings together with host aphids (*Rhopalosiphum padi)*.

Five concentrations of Sunergist (438, 875, 1750, 3500, 7000 mL product/ha), a reference item and a control, had been used for the test. Reproduction of *Aphidius rhopalosiphi* was assessed in the control and at all test item dose rates.

Mortality was recorded approximately 2, 24 and 48 hours after test initiation. The number of parasitoids alive, affected, moribund and dead was recorded. Moribund parasitoids were counted as dead. For reproduction the number of aphid mummies was counted 11-12 days after the 24 hours parasitisation period in all replicates where the females were alive after the 24 hours parasitisation period (n=18-20). Reproduction was performed where the corrected mortality was ≤ 50 %.

Mortality data were analysed for significance using the Fisher's Exact Test. For the analysis of the test item data the Bonferroni correction was applied (Bonferroni-Holm Fisher's Exact Test). Reproduction data were tested for normal distribution and homogeneity of variance using the Shapiro-Wilk's test (α = 0.05) and the Levene's test (α = 0.05). Because reproduction data were normally distributed and homogeneous, the Dunnett's t-test (multiple comparison, one-sided, α = 0.05) was used. The software used to perform the statistical analysis was ToxRat Professional, Version 3.2.1, ® ToxRat Solutions GmbH.

II. RESULTS AND DISCUSSION

Validity criteria:

- In order for the study to be valid, the control mortality should not exceed 13 %. In this study, it was 2.5 % and the validity criterion was met.
- The reference item mortality should be at least 50 % and was found to be 100.0 %, therefore, the validity criterion was met.
- For reproduction, the control reproduction rate should be ≥ 5 mummies per female and there should be no more than 2 parasitoids producing zero values. In this study, the mean number of mummies per female was 23.9 and there was no parasitoid producing zero values.

Thus, all validity criterions had been met and the study can be considered as valid.

The effect of Sunergist (Epibrassinolide 0.01% Soluble Liquid) on the mortality and on the parasitisation rate of adult *Aphidius rhopalosiphi* is summarised in Table 9-21. Because of the observed mortality, the LR_{50} after 48 h exposure was calculated to be >7000 mL product/ha (corresponding to > 0.69 g a.s./ha). Reproduction of *Aphidius rhopalosiphi* was assessed in the control and all treatment groups. The test showed no effect on the reproduction rate up to and including 7000 mL product /ha (corresponding to 0.69 g a.s./ha).

Table 9-3: Summary of effects of Sunergist (Epibrassinolide 0.01% Soluble Liquid) on adult *Aphidius rhopalosiphi* after 48 h exposure

Test item [mL product/ha]	Test rate [g a.s./ha]	Mean mortality [%]	Corrected mortality [%]	Mummies per female	Reduction of parasitisation efficiency [%]
control	0	2.5 ± 5.0	-	23.9 ± 9.1	-
438	0.043	17.5 ± 22.2	15.4	22.8 ± 14.0	4.6
875	0.087	5.0 ± 10.0	2.6	29.6 ± 16.6	-23.9
1750	0.17	2.5 ± 5.0	0.0	20.3 ± 18.8	14.7
3500	0.35	2.5 ± 5.0	0.0	20.4 ± 15.6	14.7
7000	0.69	20.0 ± 18.3	17.9	20.6 ± 16.1	13.6
Reference item: 0.3 ml Perfekthion/ha		100 ± 0.0*	100*	-	-

* significantly different from control (Fisher's Exact Test, α = 0.05)
n.d. not determined
negative values indicated better performance compared to control

III. CONCLUSIONS

Under worst case laboratory conditions the LR_{50} of Sunergist (Epibrassinolide 0.01% Soluble Liquid) is estimated to be greater than 7000 mL product/ha in 200 L water/ha (corresponding to > 0.69 g a.s./ha).
Reproduction of *Aphidius rhopalosiphi* was assessed in the control and at all test item dose rates. Reproduction was not affected up to and including 7000 mL product/ha, and the ER_{50} is therefore estimated to be greater than 7000 mL product/ha in 200 L water/ha (corresponding to > 0.69 g a.s./ha).

Effects on *Aphidius rhopalosiphi* (Moll, M., 2017)	**Comment RMS:** The study is considered relevant and reliable. The validity criteria according to Mead-Briggs *et al.* (2000) are met, no deviations occurred. The toxicity endpoints are confirmed by RMS: **LR_{50} (mortality) > 7000 mL product/ha (equivalent to > 0.69 g a.s./ha)** **ER_{50} (reproduction) > 7000 mL product/ha (equivalent to > 0.69 g a.s./ha)**

B.9.5.2.2. Effect study on *Typhlodromus pyri*

Data point addressed:	CP B.9.5.2
Reference:	KCP 10.3.2.1/0001
Author(s) (year):	Moll, M.(2017)
Title:	Sunergist (Epibrassinolide 0.01% Soluble Liquid): Effects on the Predatory Mite *Typhlodromus pyri* in the Laboratory - Dose Response Test -
Laboratory report / project Number (Doc No.):	120141063
Testing facility:	Ibacon GmbH, Rossdorf, Germany
Published:	No
Test guideline used:	Blümel et al., 2000
Deviations:	No relevant deviations
GLP:	Yes
Acceptability:	**Yes**

Executive Summary

The effect of a SL formulation containing 0.01 % Epibrassinolide applied at 5 dose rates (438 - 7000 mL product/ha, corresponding to 0.043 g a.s/L – 0.69 g a.s./ha, as 0.01% Epibrassinolide parallel 0.099 g/L Epibrassinolide) on the predatory mite *Typhlodromus pyri* was measured in the laboratory via contact on treated glass surfaces compared to a water treated control and to a reference item. An assessment on mortality seen over 48 h of exposure and for sublethal effects compared to a water treated control was performed.
Under worst case laboratory conditions the LR_{50} of Sunergist (Epibrassinolide 0.01% Soluble Liquid) for mortality effects is 2832 mL product/ha in 200 L water/ha (corresponding to 0.28 g a.s./ha)
Reproduction of *Typhlodromus pyri* was assessed in the control and at 438, 875, 1750 and 3500 mL product/ha. Reproduction was statistically significantly affected at 438, 875 and 1750 mL product/ha. The effect on reproduction was < 50 % at these dose rates. At 3500 mL product/ha reproduction was not statistically significantly affected.
The ER_{50} for reproduction effects is estimated be greater than 3500 mL product/ha in 200 L water/ha, the highest dose rate where reproduction was tested (corresponding to > 0.35 g a.s./ha).

I. MATERIALS AND METHODS

A. MATERIALS

1. Test Material:	Sunergist (Epibrassinolide 0.01% Soluble Liquid)
Active ingredient:	24-Epibrassinolide
Description:	colorless

Lot/Batch #:	002-20150506
Content of a.s.:	0.01% (w/w)

2. Vehicle and control:

Control:	Deionised water
Reference Item	Perfekthion (BAS 152 11 I) (420.3 g/L dimethoate)

3. Test animals:

Species:	*Typhlodromus pyri* Scheuten
Taxonomic group:	Predatory mites (Acari: Phytoseiidae)
Life Stage:	Protonymphs
Age:	No more than 24 hours
Source:	Katz Biotech AG, An der Birkenpfuhlheide 10, D-15837 Baruth
Acclimation period:	Under test conditions
Environmental conditions:	
Temperature:	24 – 25 °C
Humidity:	66 – 77 %
Photoperiod:	16 hours light – 8 hours dark
Light intensity:	420 – 780 lux

In life dates: 10.01.2017 – 24.01.2017

B. STUDY DESIGN AND METHODS

Exposure of the predatory mite *T. pyri* was reached via air dried residues on treated glass plates at a spraying volume of 200 L/ha. The dilutions were sprayed onto glass plates and dried for 30 to 40 minutes, before introduction of the test animals. Seven treatment groups (five test item treatment groups, water treated control, reference item) were tested with 3 replicates each and each containing 20 mites. Mortality was assessed after 3 and 7 days of exposure.
For the reproduction assessment the sex-ratio for reproduction testing was 1 male : 5 females at a minimum on day 7. If the sex-ratio was less than 1 male : 5 females, males originating from another replicate from the same treatment were added until an appropriate sex-ratio was reached on day 7. Number of eggs laid and number of live and dead juvenile stages per female was counted and removed afterwards on 3 assessment days from day 7 on with a maximum interval of 3 days up to day 14 (inclusive). Eggs laid until day 7 inclusive were removed from the test arena and were not counted.
The reproduction assessment was performed were the corrected mortality (Mcorr) was ≤ 56 %.
No reproduction assessment was performed for the reference item.

The LR_{50} of the mortality was calculated by applying the Probit-Analysis. Mortality data were analysed for significance using the Fisher's Exact Test. For the analysis of the test item data the Bonferroni correction was applied (Bonferroni-Holm Fisher's Exact Test).
Reproduction data were tested for normal distribution and homogeneity of variance using the Shapiro-Wilk's test ($\alpha = 0.05$) and the Levene's test ($\alpha = 0.05$). Because reproduction data were normally distributed and homogeneous, the Dunnett's t-test (multiple comparison, one-sided, $\alpha = 0.05$) was used.
The software used to perform the statistical analysis was ToxRat Professional, Version 3.2.1, ® ToxRat Solutions GmbH.

II. RESULTS AND DISCUSSION

Validity criteria:

- In order for the study to be valid, the control mortality should not exceed 20 % on day 7. In this study, it was 16.7% and the validity criterion was met.
- The reference item mortality should be at least 50 % and was found to be 94.0 %, therefore, the validity criterion was met.
- For reproduction, the number of eggs per female in the control should be ≥ 4 eggs for the second week. In this study, the mean number of eggs per female was 6.1.

Thus, all validity criterions had been met and the study can be considered as valid.

The effect of Sunergist (Epibrassinolide 0.01% Soluble Liquid) on the mortality of *Typhlodromus pyri* is summarised in Table 9-22. The LR_{50} after 7 day exposure was calculated to be 2832 mL product/ha (corresponding to 0.28 g a.s./ha). The 95 % confidence limit is 2124 - 4086 mL product/ha.

Reproduction of *Typhlodromus pyri* was assessed in the control and at 438, 875, 1750 and 3500 mL product/ha and is summarised in Table 9-23. Reproduction was statistically significantly affected at 438, 875 and 1750 mL product/ha. The effect on reproduction was < 50 % at these dose rates. At 3500 mL product/ha reproduction was not statistically significantly affected.
The ER_{50} for reproduction effects is estimated be greater than 3500 mL product/ha (corresponding to 0.35 g a.s./ha), the highest dose rate where reproduction was tested.

Table 9-4: Summary of mortality of Sunergist (Epibrassinolide 0.01% Soluble Liquid) on nymph *Typhlodromus pyri* after 7 days exposure

Test item [mL product/ha]	**Test rate [g a.s./ha]**	**Mean mortality [%]**	**Corrected mortality [%]**	**Escapees [%]**
control	0	16.7 ± 5.8	-	0.0 ± 0.0
438	0.043	33.3 ± 10.4	20.0	1.7 ± 2.9
875	0.087	30.0 ± 13.2	16.0	3.3 ± 5.8
1750	0.17	56.7 ± 7.6*	48.0	1.7 ± 2.9
3500	0.35	63.3± 2.9*	56.0	13.3 ± 10.4
7000	0.69	71.7 ± 2.9*	66.0	8.3 ± 7.6
Reference item		95 ± 5.0*	94.0	5.0 ± 5.0

* significantly different from control (Bonferroni-Holm Fisher's Exact Test, $\alpha = 0.05$)

Table 9-5: Summary of reproduction effect of Sunergist (Epibrassinolide 0.01% Soluble Liquid) on adult *Typhlodromus pyri*

Test item [mL product/L]	**Test rate [g a.s./ha]**	**Reproduction [eggs per female]**	**Effect on reproduction [%]**
control	0	6.1 ± 0.9	-
438	0.043	3.4 ± 0.9*	44.6
875	0.087	3.6 ± 0.4*	41.8
1750	0.17	3.7 ± 0.9*	39.3
3500	0.35	5.1 ± 1.4	16.5

* significantly different from control (Dunnett´s t-test, $\alpha = 0.05$)

III. CONCLUSIONS

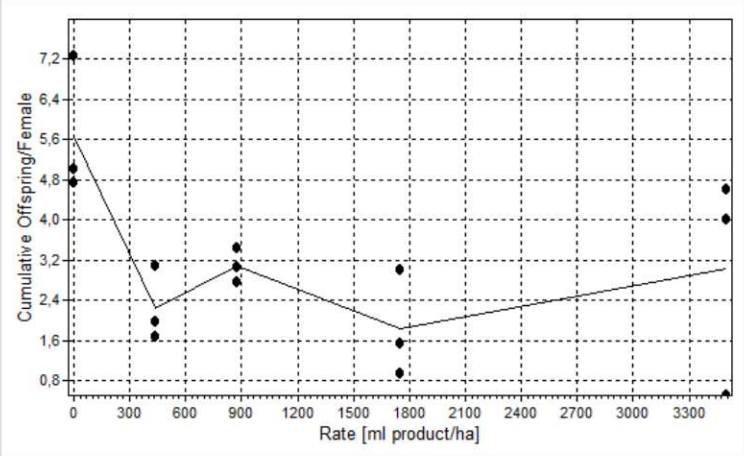
Under worst case laboratory conditions the LR_{50} of Sunergist (Epibrassinolide 0.01% Soluble Liquid) for mortality effects is 2832 mL product/ha in 200 L water/ha (corresponding to 0.28 g a.s./ha).
Reproduction of *Typhlodromus pyri* was assessed in the control and at 438, 875, 1750 and 3500 mL product/ha. Reproduction was statistically significantly affected at 438, 875 and 1750 mL product/ha. The effect on reproduction was < 50 % at these dose rates. At 3500 mL product/ha reproduction was not statistically significantly affected.
The ER_{50} for reproduction effects is estimated be greater than 3500 mL product/ha in 200 L water/ha, the highest dose rate where reproduction was tested (corresponding to > 0.35 g a.s./ha).

KCP 10.3.2.1/0001	Comment RMS: The study is considered relevant and reliable. The validity criteria according to Blümel *et al.* (2000) are met, no deviations occurred. The toxicity endpoints are recalculated by RMS using ToxRat Pro (Version 3.2.1.) with default settings: **LR_{50} (mortality, 0 - 7 d) = 2831.9 mL product/ha (95%-CL: 2124.4 – 4086.5), equivalent to 0.281 g a.s./ha.** **NOER (mortality, 0 - 7 d) < 438.0 mL product/ha (equivalent to < 0.043 g a.s./ha).** **The values for mortality and escapees in Table 9-22 are confirmed.** **No reliable ER_{50} (reproduction) could be determined, no dose-response relation for**

reduction of cumulative offspring/female is found.

%Reduction of cumulative offspring/female caused by the test item after 14 d.

Treatm.[ml produc	Mean	Std. Dev.	n	%Reduction
Control	5,7	1,39	3	
438,0	2,2	0,75	3	60,5
875,0	3,1	0,34	3	45,6
1750,0	1,8	1,06	3	67,9
3500,0	3,0	2,21	3	46,5



NOER (reproduction) < 438.0 mL product/ha (equivalent to < 0.043 g a.s./ha), significant effects were found in all test item concentrations using the Williams Multiple Sequential t-test Procedure (software default test)

Treatm. [ml produ	Mean	s	df	LhM	%MDD	t	t*	Sign.
Control	5,7	1,32						
438,0	2,2	1,32	10	2,7	-34,3	-2,81	-1,81	+
875,0	3,1	1,32	10	2,7	-36,1	-2,81	-1,91	+
1750,0	1,8	1,32	10	2,4	-36,7	-3,02	-1,94	+
3500,0	3,0	1,32	10	2,4	-37	-3,02	-1,96	+

+: significant; -: non-significant
The NOER is lower than 438,0 ml product/ha.

Validity criteria were fulfilled:
- Control mortality on day 7 < 20% : Actually 16.7%
- Minimum mean cumulative egg number/female (day 7 – 14) in the control ≥ 4: Actually 5.7
- Cumulative mean mortality (control corrected) of the reference item group on day 7 should range between 50 - 100%: Actually 94%

Table 9.5-6: 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.[1]	Moll, M. (2017)
Additional species					
No additional studies submitted.					

n.d. … not determined

[1] please refer to the study evaluation under point B.9.5.2.2

B.9.6. RISK ASSESSMENT FOR ARTHROPODS

Most insects depend on plant steroids such as brassinosteroids as source of cholesterol, which is then converted to ecdysone, a hormone regulating gene expression e.g. for larval molting, adult leg morphogenesis and cuticle production (Thummel & Chory, 2002[11]). Brassinosteroids and 24-Epibrassinolide is therefore an essential part of arthropod's diet and arthropods are naturally exposed to these substances via food.
In addition, 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. Therefore 24-Epibrassinolide is considered pose a low risk to non-target arthropods. Nonetheless risk assessments were conducted below.

B.9.6.1. Risk assessment for honey bees

24-Epibrassinolide is naturally found in various plants, pollen and honey. According to Ikekawa *et al.* (1988)[12], the highest concentration of 24-Epibrassinolide in bee pollen of *Vicia faba* was 5 µg/kg fresh weight and Khripach *et al.* (2013)[13] reported 7.4 ng/g 24-Epibrassinolide in honey. Honeybees may be exposed to formulated 24-Epibrassinolide by direct spraying of the plant protection product while bees are foraging on flowers and weeds present in or adjacent to the crop treated. They may also be exposed through contact with fresh or dry residues or by oral uptake of contaminated pollen, nectar and honey dew.

An acute oral and contact honeybee toxicity study with 24-Epibrassinolide (TGAI) was conducted. 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).**

[11] KCA 8.3/0001: Thummel, C.S., Chory, J. (2002): STEROID SIGNALING IN PLANTS AND INSECTS - COMMON THEMES, DIFFERENT PATHWAYS, Report No.: na (092-114), Genes & Development, 2002, 16, 3113-3129; DOI: 10.1101/gad.1042102, Not GLP, published

[12] KCA 8.3.1/0002: Ikekawa, N., Nishiyama, F., Fujimoto, Y. (1988): IDENTIFICATION OF 24-EPIBRASSINOLIDE IN BEE POLLEN OF THE BROAD BEAN, VICIA FABA L., Report No.: na (092-027), Chemical and Pharmaceutical Bulletin, 1988, 36 (1), 405-407, Not GLP, published

[13] KCA 8.3.1/0003: Khripach, V.A., Litvinovskaya, R.P., Kurtikova, A.L., Drach, S.V., Pryadko, A.G., Mirantsova, T.V., Baranovskiy, A.V. (2013): ENZYME IMMUNOASSAY OF THE CONTENT OF ENDOGENOUS BRASSINOSTEROIDS IN PHYTOGENIC FOOD PRODUCTS, Report No.: na (092-030), National Academy of Sciences of Belarus, 2013, 57 (2), 63-69, Not GLP, published

Acute risk assessment

Table 9.6-1: Summary of acute toxicity of 24-Epibrassinolide to honeybees

Species	Test substance	Time scale/type of endpoint	End point	Toxicity	Reference
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)

Acute oral toxicity - Screening Step (according to EFSA Bee GD 2013)

The acute exposure-toxicity (ETR) ratio for adult honey-bees was calculated using the following formula:

$$ETR_{acute\ oral\ (adult)} = \frac{AR\ x\ SV}{LD_{50\ oral}}$$

Where: AR…Application rate [kg a.s./ha], SV…Shortcut value, LD_{50}…Lethal dose [µg a.s./bee]

Table 9.6-2: Risk to honeybees from acute oral exposure to 24-Epibrassinolide following the use of Sunergist in grapes (3 x 0.05 g a.s./ha), covering all other uses

Crop	Application rate	Test substance	Endpoint	SV	$ETR_{acute\ oral\ (adult)}$	Trigger
Grapes	0.00005 kg a.s./ha	24-Epibrassinolide	LD_{50} > 92.2 µg a.s./bee	10.6[1]	< 0.000006	0.2

Values in **bold** are above the trigger value

[1] Application made via sideward spraying

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.

Acute contact toxicity – Screening Step (according to EFSA Bee GD 2013)

The contact Hazard Quotient was calculated using the following formula:

$HQ_{contact} = AR / LD_{50\ contact}$

Where: AR…Application rate [g a.s./ha], LD_{50}…Lethal dose [µg a.s./bee]

Table 9.6-3: Risk to honeybees from acute contact exposure to 24-Epibrassinolide following the use of Sunergist in grapes (3 x 0.05 g a.s./ha), covering all other uses

Crop	Application rate	Test substance	Endpoint	$HQ_{contact}$	Trigger
Grapes	0.05 g a.s./ha	24-Epibrassinolide	LD_{50} > 10.0 µg a.s./bee	< 0.005	85[1]

[1] Trigger for sideward spray application

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.

Calculation of HQ_O and HQ_C according to Terrestrial Ecotoxicology GD (SANCO/10329/2002)

The hazard quotients (EPPO/OEPP (2003) Environmental risk assessment scheme for plant protection products, Chapter 10: Honeybees (PP 3/10(2)). Bulletin OEPP/EPPO Bulletin 33: 141-145.) were calculated as follows:

$$\text{Hazard Quotient} = \frac{\text{Maximum application rate [g a.s./ha]}}{\text{Acute } LD_{50} \text{ [µg/bee]}}$$

A hazard quotient of less than 50 indicates a low risk to bees in the field.

The resulting QH_O and QH_C values are presented in Table 9.6-4.

Table 9.6-4: Hazard quotient for oral toxicity to honeybees following 24-Epibrassinolide exposure after the use of Sunergist in grapes (3 x 0.05 g a.s./ha), covering all other uses

Test item	Use pattern	Exposure route	Max. single application rate	Endpoint LD_{50} [µg a.s./bee]	Hazard quotient (HQ)	Trigger
24-Epibrassinolide	Foliar spray	Oral	0.05 g a.s./ha	> 92.2	< 0.0005	50
24-Epibrassinolide	Foliar spray	Contact	0.05 g a.s./ha	> 10	< 0.005	50

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.

Chronic risk assessment

No studies were submitted by the notifier 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.

The applicant submitted the following statement:
"Due to the ubiquitous occurrence of 24-Epibrassinolide and other brassinosteroids in plants, nectar and pollen, the natural exposure of bees to the substance, as well as the low acute oral and acute contact toxicity for bees, testing of chronic toxicity to bees is not considered necessary. Further, none of the co-formulants is considered toxic to bees. Further, in product studies with non-target arthropods, an acceptable risk to these organisms was found. Therefore, also the chronic risk to bees from the application of Sunergist is considered low."

This argument is only partly accepted by RMS. There is evidence, that 24-Epibrassinolide is naturally found in various plants, pollen and honey. According to Ikekawa *et al.* (1988)[14], the highest concentration of 24-Epibrassinolide in bee pollen of *Vicia faba* was 5 µg/kg fresh weight and Khripach *et al.* (2013)[15] reported 7.4 ng 24-Epibrassinolide/g in honey.
The public literature by Chuda-Mickiewicz *et al.* (2009, reference KCA 8.3.1/0001, evaluated in Vol. 3 CA B9 B.9.3.1) 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.

[14] KCA 8.3.1/0002: Ikekawa, N., Nishiyama, F., Fujimoto, Y. (1988): IDENTIFICATION OF 24-EPIBRASSINOLIDE IN BEE POLLEN OF THE BROAD BEAN, VICIA FABA L., Report No.: na (092-027), Chemical and Pharmaceutical Bulletin, 1988, 36 (1), 405-407, Not GLP, published

[15] KCA 8.3.1/0003: Khripach, V.A., Litvinovskaya, R.P., Kurtikova, A.L., Drach, S.V., Pryadko, A.G., Mirantsova, T.V., Baranovskiy, A.V. (2013): ENZYME IMMUNOASSAY OF THE CONTENT OF ENDOGENOUS BRASSINOSTEROIDS IN PHYTOGENIC FOOD PRODUCTS, Report No.: na (092-030), National Academy of Sciences of Belarus, 2013, 57 (2), 63-69, Not GLP, published

In Table 9.6-5 and Table 9.6-6 an estimation of 24-Epibrassinolide uptake by adult bees and bee larvae via nectar is presented (exposure via pollen consumption was not considered because the pollen uptake is lower than the nectar consumption), and the chronic endpoints required to achieve an acceptable risk are shown for better illustration.

Table 9.6-5: Estimation of exposure via nectar uptake in adult bees

	Residue in nectar [ng a.s./mg nectar]	**Nectar consumption**[3] [mg nectar/bee/day]	**Uptake of a.s. via nectar consumption [µg a.s./bee/day]**	**Acute oral toxicity [µg a.s./bee]**
EPPO default	1[1]	853.3	0.853	LD_{50} > 92.2
Khripach *et al.* (2013)[4]	0.05[2]	853.3	0.043	

[1] default worst case residue content is 1 mg a.s./kg nectar or pollen according to EPPO (2010).
[2] Worst case assumption by RMS: 5 µg a.s./kg pollen x 10 (assuming nectar contains 10 x more 24-Epibrassinolide than pollen).
[3] 128 mg sugar/bee/day, where nectar contains 15% sugar (i.e. 128/0.15 = 853.3 mg nectar/bee/day), according to EFSA Bee GD (2013).
[4] for full reference see previous page.

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.

Table 9.6-6: Estimation of exposure via nectar uptake in bee larvae

	Residue in nectar [ng a.s./mg nectar]	**Nectar consumption**[3] [mg nectar/larva/5 days]	**Uptake of a.s. via nectar consumption [µg a.s./larva/5 days]**
EPPO default	1[1]	396	0.396
Khripach *et al.* (2013)[4]	0.05[2]	396	0.020

[1] default worst case residue content is 1 mg a.s./kg nectar or pollen according to EPPO (2010).
[2] Worst case assumption by RMS: 5 µg a.s./kg pollen x 10 (assuming nectar contains 10 x more 24-Epibrassinolide than pollen).
[3] 59.4 mg sugar/larva/5 days, where nectar contains 15% sugar (i.e. 59.4/0.15 = 396 mg nectar/larva/5 days), according to EFSA Bee GD (2013).
[4] for full reference see footnote 15 on the previous page.

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 by the applicant 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.

Risk assessment for exposure to contaminated water (according to EFSA Bee GD 2013)

The risk to honeybees posed by the exposure to contaminated water was assessed with the EFSA Bee Tool calculator v.3. The input parameter and the results of the screening step are presented in the tables below. As previously mentioned, the new EFSA Bee GD (2013) is not yet noted by the Member States and therefore this risk assessment is only shown for informative purposes (i.e. not conclusive for authorization).

Table 9.6-7: Input parameters for the risk assessment of honeybees exposed to contaminated water

Input parameter	
Application rate (g a.s./ha)	0.05
Water solubility (mg/L)	3.8[1]
PEC_{SW} (mg a.s./L)	0.0000232[2]
Contact LD_{50} (µg a.s./bee)	> 10.0
Oral LD_{50} (µg a.s./bee)	> 92.2
Chronic adult	No data
Larvae	No data
HPG	No data

[1] Please refer to Vol. 3 CA Part B 2

[2] Maximum PECsw of FOCUS Step 2 of cucurbits (Northern Europe, Oct-Febr), 0.0232 µg a.s./L

Guttation

Table 9.6-8: First tier risk assessment of guttation fluid for the critical use of 3 x 0.05 g a.s./ha in grapes (covering all other uses)

Is the substance applied after the guttation period? → Unclear, go to next question.					
Does guttation water occur for less than 10% of the location/calendar year combinations? → Unclear, go to 1st tier.					
1st tier for guttation					
Application rate (g a.s./ha)		**water consumption (µL)**	**ETR**	**Trigger**	**Risk indicator**
0.05	acute	11.4	0.0	0.2	OK
0.05	chronic	11.4	No data	0.03	No data
0.05	larvae	111	No data	0.2	No data
0.05	HPG	11.4	No data	1	No data

Values in **bold** are above the trigger value

After the first tier assessment of guttation water exposure the acute ETR is below the trigger value of 0.2 for the use of Sunergist in grape (which covers all other uses). Therefore the risk posed by 24-Epibrassinolide in guttation water is considered acceptable.

Surface water

Table 9.6-9: Screening step risk assessment of surface water for the critical use of 3 x 0.05 g a.s./ha in grapes (covering all other uses)

Surface water				
	water consumption (µL)	**ETR**	**Trigger**	**Risk indicator**
acute	11.4	0.00	0.2	OK
chronic	11.4	No data	0.03	No data
larvae	111	No data	0.2	No data
HPG	11.4	No data	1	No data

The acute ETR is below the trigger value of 0.2, hence an acceptable risk to honeybees posed by surface water can be concluded.

Puddle water

Table 9.6-10: Screening step risk assessment of puddle water for the critical use of3 x 0.05 g a.s./ha in grapes (covering all other uses)

Puddle water				
	water consumption (µL)	**ETR**	**Trigger**	**Risk indicator**
acute	11.4	0.00	0.2	OK
chronic	11.4	No data	0.03	No data
larvae	111	No data	0.2	No data
HPG	11.4	No data	1	No data

The acute ETR is below the trigger value of 0.2, hence an acceptable risk to honeybees posed by puddle water can be concluded.

B.9.6.2. Risk assessment of non-target arthropods other than bees

Non-target arthropods may be exposed to formulated 24-Epibrassinolide in-field by direct spraying of the plant protection product or via drift of the product into off-field adjacent to the treated crop. 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.

Table 9.6-11: Laboratory toxicity endpoints with standard sensitive non-target arthropod species used in the risk assessment

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.[1]	Moll, M. (2017)
Additional species					
No additional studies submitted.					

n.d. … not determined

[1] please refer to the study evaluation under point B.9.5.2.2

In-field risk assessment

The potential risk of 24-Epibrassinolide to in-field non-target arthropods was assessed by calculation of the hazard quotient (HQ = exposure/toxicity) according to the following formula:

$$HQ_{in-field} = \frac{Application\ rate\ x\ MAF}{LR_{50}}$$

The default ESCORT 2 MAF of 2.3 for foliar application was used.

Table 9.6-12: In-field HQs for the application of Sunergist (0.01% 24-Epibrassinolide SL) in grapes (3 x 0.05 g a.s./ha), covering all other uses

Species	Application rate (g a.s./ha)	MAF	LR_{50} (g a.s./ha)	$HQ_{in\text{-}field}$	Trigger
Aphidius rhopalosiphi Tier I, 2D exposure scenario	0.05	2.3	> 0.69	< 0.17	2
Typhlodromus pyri Tier I, 2D exposure scenario	0.05	2.3	0.28	0.41	2

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.

Off-field risk assessment

In order to assess the potential risk of 24-Epibrassinolide to off-field non-target arthropods following the use of Sunergist, the foliar predicted environmental rate is compared with the toxicity endpoints according to the following formula:

$$HQ_{off-field} = \frac{Application\ rate\ x\ MAF\ x\ \left(\frac{drift\ factor}{VDF}\right)}{LR_{50}}\ xcorrection\ factor$$

Where: drift factor = % drift/100; VDF… vegetation distribution factor

The worst case drift factor of 6.9 % (3 m distance, 3 applications, late application) according to ESCORT 2 for vineyards was used covering the representative uses in leaf vegetables and sugar beet. A vegetation distribution factor of 2 is used in the risk assessment along with the MAF of 2.3 and a correction factor of 10.

Table 9.6-13: Off-field HQs for the application of Sunergist (0.01% 24-Epibrassinolide SL) in grapes (3 x 0.05 g a.s./ha), covering all other uses

Species	App. rate (g a.s./ha)	MAF	Drift factor	VDF	LR_{50} (g a.s./ha)	Correction factor	$HQ_{off\text{-}field}$	Trigger
Aphidius rhopalosiphi Tier I, 2D exposure	0.05	2.3	0.069	2	> 0.69	10	< 0.06	2
Typhlodromus pyri Tier I, 2D exposure	0.05	2.3	0.069	2	0.28	10	0.14	2

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.

B.9.7. EFFECTS ON NON-TARGET SOIL MESO- AND MACROFAUNA

B.9.7.1. Earthworms

Active substance data

No active substance laboratory studies were submitted by the notifier to address the sub-effects on earthworms, please refer to Vol. 3 CA B9 B.9.4.1.

B.9.7.1.1. Sub-lethal effects to earthworms

Product data

No laboratory studies were submitted by the notifier to address the sub-effects on earthworms following the exposure to the formulated product. RMS considers the waiving of the sub-lethal effect study with earthworms acceptable by taking the natural background concentration and natural occurrence of brassinosteroids in soil into account. Negative effects posed by 24-Epibrassinolide are considered unlikely and a qualitative risk consideration is presented below under point B.9.8.1.

B.9.7.1.2. Field studies with earthworms

Product data

No negative effects on earthworms from the use of Sunergist according to GAP are expected (please refer to Vol. 3 B9 B.9.4.1). Therefore, no studies were considered necessary.

B.9.7.2. Effects on non-target soil meso- and macrofauna (other than earthworms)

Active substance and product data

No laboratory studies with the active substance or the formulation were submitted by the notifier to address the effects on non-target soil meso- and macrofauna other than earthworms. Please refer to B.9.7.1 and Vol. 3 CA B9 B.9.4.2. RMS considers the waiving of the studies acceptable by taking the natural background concentration and natural occurrence of brassinosteroids in soil into account. Negative effects posed by 24-Epibrassinolide are considered unlikely and a qualitative risk consideration is presented below under point B.9.8.1 and B.9.8.2.

B.9.7.2.1. Species level testing

Not considered necessary, please refer to B.9.7.2.

B.9.7.2.2. Higher tier testing

Not considered necessary, please refer to B.9.7.2.

B.9.8. RISK ASSESSMENT FOR NON-TARGET SOIL MESO- AND MACROFAUNA

B.9.8.1. Risk assessment for earthworms

No laboratory studies were submitted by the notifier to address the sub-lethal effects on earthworms following the exposure to the active substance or the formulated product. Instead the following statement was provided by the applicant:

"Earthworms are naturally exposed to Brassinosteroid-containing plant materials such as roots, litter and other plant parts and thus there is a constant natural intake of Brassinosteroids via earthworm feed. Due to the fast uptake of Brassinosteroids by plants when coming in contact with soil and the rapid degradation ($DT_{50} \leq 43.3$ d, see [Vol. 3 CA B8, the DT_{50} of ≤ 43.3 d was not accepted by RMS in the fate section]*) as well as the low predicted environmental concentration in soil (PEC_{soil}=0.18 µg/kg soil, see* [Vol. 3 CP B8]*) no effects from the use of 24-Epibrassinolide according to GAP are expected on earthworms. Further, the co-formulants of the formulation Sunergist are not toxic to earthworms (see* [Vol. 4 Part C]*). This is why no risk assessment is considered necessary."*

RMS agrees in general to this argumentation and additionally carried out a qualitative risk consideration:

No explicit data was presented by the applicant regarding natural background concentrations of 24-Epibrassinolide in soil. However in the Fate section (Vol. 3 CA B.8.1.1.1) it is referenced, that Heumann *et al.* (2011)[16] studied the phytosterol content in soil samples. The samples were taken from different soil types such as podzoles, gleysoles cambisols and intermediates. In the measured soil samples the overall sterol concentrations ranged between 100 and 3600 mg/kg soil, the concentrations of the brassinosteroid precursor β-sitosterol ranged from 1 – 100 mg/kg soil. Due to the relative structural similarity the referenced sterols are considered suitable to serve as a proxy to estimate the order of magnitude of 24-Epibrassinolide concentration in soil.

The following worst case PECsoil values were calculated in the Fate section:

Table 9.8-1: Worst case PECsoil calculation for grapes (covers all other applications)

Crop	Application	Crop interception	DT_{50} [d]	Max. PECsoil [mg a.s./kg soil]
Grapes	3 x 0.05 g a.s./ha	0% [1]	69.02 [1]	0.0002

[1] worst case assumption, for details please refer to Vol. 3 CP B.8

The maximum application rate of 3 x 0.05 g a.s./ha in grapes results in a PECsoil of 0.0002 mg a.s./kg soil (with worst case assuptions of 0 % crop interception and a $DT_{50\ soil}$ of 69.02 days) and covers the GAP of all other uses. This maximum worst case PECsoil of 0.0002 mg a.s./kg soil is around ~ 5000 times below the lowest reported soil sterol concentrations (i.e. 1 mg β-sitosterol/kg soil reported by Heumann *et al.*, 2011). This supports that the natural exposure of earthworms to brassinosteroids (including 24-Epibrassinolide) in soil can be considered to be higher than the exposure following an application of the active substance in the form of a plant protection product.
Moreover free phytohormones such as 24-Epibrassinolide in soil are considered to be readily taken up by plants via roots.[17] Thus, it is to be expected that in case brassinosteroids occur freely in soil, e.g. if brassinosteroids are released by degradation of organic plant matter or during the use of brassinosteroid-containing plant protection products, brassinosteroids are taken up by roots and subsequently metabolised by plants.

Overall, RMS concludes that the risk to earthworms posed by the application of Sunergist following the proposed GAP can be considered acceptable and no further risk assessment is required.

B.9.8.2. Risk assessment for non-target soil meso- and macrofauna (other than earthworms)

No studies were submitted to address effects on non-target soil meso- and macrofauna (other than earthworms) following exposure of the active substance or the formulated product. Due to the GAP an exposition of soil dwelling organisms can't be excluded, however a low acute toxicity for bees and non-target arthropods other than bees was demonstrated.

The following statement was provided by the applicant:
"Other non-target soil meso- and macrofauna is naturally exposed to Brassinosteroid-containing plant materials such as roots, litter and other plant parts and thus there is a constant natural intake of Brassinosteroids via their feed. Due to the fast uptake of Brassinosteroids by plants when coming in contact with soil and the rapid degradation ($DT_{50} \leq 43.3$ d, see [Vol. 3 CA B8, the DT_{50} of ≤ 43.3 d was not accepted by RMS in the fate section]*) as well as the low predicted environmental concentration in soil (PEC_{soil}=0.18 µg/kg soil, see* [Vol. 3 CP B8]*) no effects from the use of 24-Epibrassinolide according to GAP are expected on other non-target soil meso- and macrofauna. Further, the co-formulants of the formulation Sunergist are not toxic to*

[16] KCA 7.1.1.1/0005: Heumann, S., Schlichting, A., Böttcher, J., Leinweber, P. (2011): Sterols in soil organic matter in relation to nitrogen mineralization in sandy arable soils, J. Plant Nutr. Soil Sci., 2011, 174, 576-586; doi: 10.1002/jpln.200900273, Not GLP, published

[17] KCA 6.2.1/0002: Nishikawa, N. Toyama, S. Shida, A. Futatsuya, F. (1994): THE UPTAKE AND THE TRANSPORT OF 14C-LABELED EPIBRASSINOLIDE IN INTACT SEEDLINGS OF CUCUMBER AND WHEAT Report No.: na (092-088) Journal of Plant Research, 1994, 107, 125-130 Not GLP, published

other non-target soil meso- and macrofauna (see [Vol. 4 Part 3]*). Thus, no risk assessment is considered necessary."*

RMS agrees in general to this argumentation and additionally carried out a qualitative risk consideration, please refer to point B.9.8.2 above. In addition, a low acute toxicity was demonstrated in the standard laboratory tests with bees (conducted with the active substance) and the non-target arthropods species *A. rhopalosiphi* and *T. pyri* (studies with the formulated product "Sunergist").

Therefore, in conclusion the waiving of the effect studies with (other than earthworms) is considered acceptable. RMS concludes that the risk to non-target soil organisms posed by the application of Sunergist following the proposed GAP can be considered acceptable and no further risk assessment is required.

B.9.9. EFFECTS ON SOIL NITROGEN TRANSFORMATION

Active substance and product data

No studies were submitted by the notifier to address the effects of the active substance or the formulated product on soil nitrogen transformation, please refer to Vol. 3 CA B9 B.9.5. RMS considers the waiving of the studies acceptable by taking the natural background concentration and natural occurrence of brassinosteroids in soil into account. Negative effects posed by 24-Epibrassinolide are considered unlikely and a qualitative risk consideration is presented below under point B.9.10.

B.9.10. RISK ASSESSMENT FOR SOIL NITROGEN TRANSFORMATION

The applicant submitted the following statement:
"No risk assessment for 24-Epibrassinolide or the formulation Sunergist on soil microbial activity is considered necessary due to the following reasons:

- *24-Epibrassinolide is ubiquitous in plant and plant material.*
- *Free 24-Epibrassinolide in soil will be absorbed readily by plants and not interact with any soil processes such as nitrogen transformation*
- *Tsavkelova et al., 2006 reported that the fungus Cercospora archidicola is a brassinosteroid producer as well as the green alga Chlorella vulgaris (see also CA 8.2.6). Brassinosteroids including 24-Epibrassinolide are therefore naturally present in soil systems and no effects on soil microbial activity are expected.*
- *No co-formulants of ecotoxicological concern are part of the formulation Sunergist (see* [Vol. 4 Part C]*)"*

RMS agrees in general to this argumentation and additionally carried out a qualitative risk consideration:

No studies were submitted by the notifier to address the effects of the active substance or the formulated product on soil nitrogen transformation. No explicit data was presented by the applicant regarding natural background concentrations of 24-Epibrassinolide in soil. The public literature study by Tsavkelova *et al.* (2006, reference KCA 8.5/0001, evaluated in Vol. 3 CA B9 B.9.5) does not provide explicit information regarding the effects on soil nitrogen transformation of brassinosteroids to soil microorganisms, but supports the argument that microorganisms are likely to be able to metabolise brassinosteroids present in soil (e.g. that common soil organisms like alage or funghi are capable of producing and cleaving steroid hormones). Although it is considered reasonable that negative effects on the soil nitrogen transformation following an exposure to 24-Epibrassinolide are unlikely, the presented study is not considered sufficiently reliable or relevant for a definitive conclusion.

However in the Fate section (Vol. 3 CA B.8.1.1.1) it is referenced, that Heumann *et al.* (2011)[18] studied the phytosterol content in soil samples. The samples were taken from different soil types such as podzoles, gleysoles cambisols and intermediates. In the measured soil samples the overall sterol concentrations ranged between 100 and 3600 mg/kg soil, the concentrations of the brassinosteroid precursor β-sitosterol ranged from 1 – 100 mg/kg soil. Due to the relative structural similarity the referenced sterols are considered suitable to serve as a proxy to estimate the order of magnitude of 24-Epibrassinolide concentration in soil.
Worst case PECsoil values were calculated in the fate section (please refer to Vol. 3 CP B 8). Following the maximum application rate of 3 x 0.05 g a.s./ha in grapes the resulting PECsoil is 0.0002 mg a.s./kg soil (with worst case assuptions of 0 % crop interception and a $DT_{50\ soil}$ of 69.02 days) and covers the GAP of all other uses. This maximum worst case PECsoil of 0.0002 mg a.s./kg soil is around ~ 5000 times below the lowest reported soil sterol concentrations (i.e. 1 mg β-sitosterol/kg soil). This supports that the natural exposure to brassinosteroids (including 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.
Moreover free phytohormones such as 24-Epibrassinolide in soil are considered to be readily taken up by plants via roots.[19] Thus, it is to be expected that in case brassinosteroids occur freely in soil, e.g. if brassinosteroids are released by degradation of organic plant matter or during the use of brassinosteroid-containing plant protection products, brassinosteroids are taken up by roots and subsequently metabolised by plants.

Overall, RMS concludes that the risk to soil nitrogen transformation posed by the application of Sunergist following the proposed GAP can be considered acceptable and no further risk assessment is required.

B.9.11. EFFECTS ON TERRESTRIAL NON-TARGET HIGHER PLANTS

Active substance and product data
No laboratory studies were submitted by the notifier to address the effects of the active substance on terrestrial non-target plants, but the public literature studies evaluated in Vol. 3 CA B9 B.9.6 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). For further details please refer to Vol. 3 CP B3.
Therefore RMS concludes 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 Sunergist following the proposed GAP are considered unlikely.

B.9.11.1. Summary of screening data

Not considered necessary, please refer to B.9.11.

B.9.11.2. Testing on non-target plants

Not considered necessary, please refer to B.9.11.

[18] KCA 7.1.1.1/0005: Heumann, S., Schlichting, A., Böttcher, J., Leinweber, P. (2011): Sterols in soil organic matter in relation to nitrogen mineralization in sandy arable soils, J. Plant Nutr. Soil Sci., 2011, 174, 576-586; doi: 10.1002/jpln.200900273, Not GLP, published

[19] KCA 6.2.1/0002: Nishikawa, N. Toyama, S. Shida, A. Futatsuya, F. (1994): THE UPTAKE AND THE TRANSPORT OF 14C-LABELED EPIBRASSINOLIDE IN INTACT SEEDLINGS OF CUCUMBER AND WHEAT Report No.: na (092-088) Journal of Plant Research, 1994, 107, 125-130 Not GLP, published

B.9.11.3. Extended laboratory studies on non-target plants

Not considered necessary, please refer to B.9.11.

B.9.11.4. Semi-field and field tests on non-target plants

Not considered necessary, please refer to B.9.11.

B.9.12. RISK ASSESSMENT FOR TERRESTRIAL NON-TARGET HIGHER PLANTS

The applicant submitted the following statement:
"As 24-Epibrassinolide is a naturally occurring plant hormone, which was reported in numerous higher plants (see [Vol. 3 CA Appendix I, Table A-1]*) and the formulation does not contain any ingredient toxic to plants (see Document J), no risk assessment for higher plants is considered necessary. This is also supported by the fact that no negative effects on plants were observed in efficacy trials with the formulation Sunergist."*

RMS agrees to this argumentation, please refer to B.9.11 for further justification.
It is concluded that the risk to non-target terrestrial plants posed by the application of Sunergist following the proposed GAP can be considered acceptable and no further risk assessment is required.

B.9.13. 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.

B.9.14. RISK ASSESSMENT FOR OTHER TERRESTRIAL ORGANISMS (FLORA AND FAUNA)

Not relevant, please refer to B.9.13.

B.9.15. MONITORING DATA

Monitoring data concerning effects of the active substance and the formulated product to non-target organisms are not available and are not a mandatory requirement.

B.9.16. REFERENCES RELIED ON

Literature search:
A literature search was performed for the active substance 24-Epibrassinolide, please refer to Vol. 3 CA B9 under point B.9.10.

List of data submitted by the applicant and relied on:

Data point	Author(s)	Year	Title Doc. No., (prev. used Doc. No.), (Report No.) Source (where different from company) GLP or GEP status, Published or not	Vertebrate study Y/N	Data protection claimed Y/N	Justification if data protection is claimed	Owner	Previously submitted Y/N
CP B.9.1.2	[redacted]	2017	ACUTE ORAL TOXICITY STUDY IN RATS WITH SUNERGIST (EPIBRASSINOLIDE 0.01% SOLUBLE LIQUID) Report No.: 6126 (526-001) [redacted] GLP, unpublished	Y	Y	New study necessary for the approval of 24-Epibrassinolide	Suntton GmbH	N
CP B.9.5.2	Moll, M.	2017	SUNERGIST (EPIBRASSINOLIDE 0.01% SOLUBLE LIQUID): EFFECTS ON THE PARASITOID APHIDIUS RHOPALOSIPHI IN THE LABORATORY - DOSE RESPONSE TEST - Report No.: 120141001 (834-001) Ibacon GmbH, Rossdorf, Germany GLP	N	Y	New study necessary for the approval of 24-Epibrassinolide	Suntton GmbH	N
CP B.9.5.2	Moll, M.	2017	SUNERGIST (EPIBRASSINOLIDE 0.01% SOLUBLE LIQUID): EFFECTS ON THE PREDATORY MITE TYPHLODROMUS PYRI IN THE LABORATORY - DOSE RESPONSE TEST - Report No.: 120141063 (834-002) Ibacon GmbH, Rossdorf, Germany GLP, unpublished	N	Y	New study necessary for the approval of 24-Epibrassinolide	Suntton GmbH	N

na = not applicable / ni = not indicated / nr = not relevant